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B. YOUNGBLOOD

Dr. Jacob Joseph Taubenhaus, for nearly 22 years Chief of the Division of Plant Pathology and Physiology, Texas Agricultural Experiment Station, and a charter member of the American Phytopathological Society, after an illness of about five months, died of heart trouble, terminating in pneumonia, on December 13, 1937.

Dr. Taubenhaus was born at Safed, Palestine, near the Sea of Galilee, October 20, 1885. His interest in agriculture began in his very early childhood. At twelve years of age, he entered the only agricultural school then in Palestine, at Mikve, Israel, conducted by the Alliance Israelite Universelle. Upon completion of his work there in 1898, he came to the United States and entered the National Farm School at Doylestown, Pennsylvania, from which he was graduated in 1904. At this School, he showed his deep interest in horticulture, which continued the rest of his life. He entered Cornell University in 1904, where he was graduated with the degree of Bachelor of Science in 1908 and Master of Science in 1909, in plant pathology and physiology. He next enrolled at the University of Pennsylvania, where he studied under Dr. J. W. Harshberger and was granted the degree of Doctor of Philosophy in 1913. His thesis, submitted in partial fulfilment, was entitled "The Diseases of the Sweet Pea."

Young Taubenhaus was ever an earnest and diligent worker. At Cornell, he was sustained by student labor, and his graduate work there was made possible by a personal loan from Dean Liberty Hyde Bailey, to whom Taubenhaus was very devoted. During his last year at Cornell he served as landscape gardener to President Andrew D. White, retired. As an undergraduate, he carried on experiments to determine the effect of ether on seed germination. He was also strongly influenced by Dr. H. H. Whetzel, under whom he became interested in the diseases of ornamental plants.

To Taubenhaus, the United States was a land of opportunity. He was fond of our economic and social institutions and of our form of government, all of which he studied closely. He became a naturalized citizen of the United States in 1908.

He became Assistant Plant Pathologist at the Delaware Agricultural Experiment Station in 1909, and, in 1913, was promoted to the rank of Associate, which position he held until 1916. Here, Taubenhaus collaborated with Dr. Melville T. Cook in the study of the possible relations of tannins to resistance to plant diseases and carried on extensive studies of the diseases of sweet potatoes. A noteworthy contribution at the time was his description of *Sclerotium bataticola* Taub.

Dr. Taubenhaus became Chief of the Division of Plant Pathology and Physiology at the Texas Agricultural Experiment Station in 1916. This position he held until the time of his death. From 1924 to 1937, he was also a member of the graduate faculty of the College.

For some years after going to the Texas Station, he did pioneer work in arousing interest in disease control. In this connection, he identified diseases and made recommendations for control to the Extension Service and identified the causes of loss in transit for the railroads. Despite these services, he carried on extensive investigations, rarely interrupted even by vacations. While devoting major attention to the study of the cotton root rot (*Phymatotrichum omnivorum*), he continued his interest in the diseases of sweet potatoes and of many other plants, particularly onions, melons, and tomatoes.

The Phymatotrichum disease study was long continued and encyclopedic. His first and most extensive publication on this subject, 1923, advanced the idea that the puzzling differences between this disease and the Fusarium and other root diseases are to be explained by the close association of living mycelium of the fungus with the roots—particularly, with partially decayed, as compared to completely decayed portions of infected roots—and by the fact that the spread of the disease is chiefly along roots rather than through the soil itself. He developed methods of inoculating plants with this disease-producing organism on a large scale, which facilitated field experiments. Inoculation experiments with monocotyledonous plants verified the conclusion that such plants are immune from the *Phymatotrichum* fungus. This fact is basic to the control measures now advocated in Texas, including sorghum in crop rotation and "sorghum barriers," which latter tend to block the advance of the disease across cotton fields. The results of his extensive studies on the host range of the Phymatotrichum root rot were embodied in a bulletin, which was completed only a few months before his final illness. He and his associates tested the relative susceptibility of more than 2,000 species and varieties of flowering plants to the Phymatotrichum root-rot fungus.

In Texas he worked with many other specialists, State and Federal. Beginning in 1927, large State appropriations for the expansion of root-rot research made possible the employment of a number of associates. In addition, he cooperated with a number of agronomists and chemists of the Texas Station in an endeavor to follow out the varied ramifications of the root-rot problem.

Dr. Taubenhaus was Russell Lecturer for the Massachusetts State Horticultural Society in 1916; and, was chairman of the committee on the preparation of exhibit entitled "The Diseases of Plants," for the Texas Centennial Central Exposition, 1936.

Dr. Taubenhaus' life was characterized by the great zeal with which he conducted his research and the large scale upon which he planned many of his experiments. Instead of using a few cultures, he used hundreds of them.

Some who knew him intimately feel that too long and continuous application to his work may have hastened his death.

Dr. Taubenhaus was a charter member of the American Phytopathological Society and had been secretary of the Southern Division; Fellow, American Association for the Advancement of Science; Texas Academy of Science (chairman, "Grants" committee); member, Botanical Society of America, Mycological Society of America, American Rose Society, Natural History Society of Delaware, Nature Association, National Association of Gardeners, Authors' League of America, Authors' Club (London), Cotton Disease Council (chairman of section on Phymatotrichum root rot); was a member of the Sigma Xi fraternity; Mason; and B'nai B'rith.

In addition to his scientific work, he took an active part in religious activities. For years he was leader of the congregation of Temple Freda in Bryan, Texas. He also served as president of the local lodge of B'nai B'rith there. On the college campus, he organized the Hillel Club composed of Jewish students and acted as their guide and adviser. During the influenza epidemic of 1918, he spent all of his spare time in the hospital aiding in nursing. In addition, he was a member of the Provisional Executive Committee for Zionist affairs.

Dr. Taubenhaus was a director for Texas, and a constant supporter, of the National Farm School, Doylestown, Pa. In 1926 he was the principal speaker and guest of honor at a banquet given in New York City for the benefit of the National Farm School at which 1,000 guests paid $1,000 per plate, the proceeds forming a million dollar endowment fund for the school.

Dr. Taubenhaus married Esther Hirschenson of Hoboken, New Jersey, in 1910—children, Leon Jahir and Ruth Ziporah. In addition to his immediate family, he is survived by his father and mother, Meyer and Bath-sheba Taubenhaus, three brothers and three sisters in Haifa, Palestine, and another brother in New York City.

His untimely death was a great loss to the State of Texas; and his stimulating presence will be greatly missed from the annual meetings of The American Phytopathological Society. He practically always attended these meetings and participated in the programs.

Dr. Taubenhaus was a prolific writer. Because of space limitations only his principal publications are listed below. Besides these, Dr. Taubenhaus, or he and his associates, also wrote on a wide range of subjects for professional and agricultural journals. He presented many papers on plant diseases before farmers' short courses, local and national agricultural and scientific societies, and was a frequent speaker over the college radio station, WTAW.

PRINCIPAL PUBLICATIONS

(List assembled by Dr. W. N. Ezekiel)

1. A contribution to our knowledge of the morphology and life history of *Puccinia malvacearum* Mont. Phytopath. **1**: 55–62. 1911.
2. Protective enzymes. (Cook, Mel T., H. P. Bassett, Firman Thompson, and J. J. Taubenhaus). Science (n.s.) **33**: 624–629. 1911.

3. The relation of parasitic fungi to the contents of the cells of the host plants (I. The toxicity of tannin). (Cook, Mel. T., and J. J. Taubenhaus). Del. Agr. Exp. Sta. Bul. 91. 1911.
4. A study of some *Gloeosporiums* and their relation to a sweet pea disease. Phytopath. **1**: 196–202. 1911.
5. The relation of parasitic fungi to the contents of the cells of the host plants (II. The toxicity of vegetable acids and the oxidizing enzymes)—(Cook, Mel T., and J. J. Taubenhaus). Del. Agr. Expt. Sta. Bul. 97. 1912.
6. A further study of some *Gloeosporiums* and their relation to a sweet pea disease. Phytopath. **2**: 153–160. 1912.
7. The black rots of the sweet potato. Phytopath. **3**: 159–166. 1913.
8. Recent studies of some new or little known diseases of the sweet potato. Phytopath. **4**: 306–320. 1914.
9. The diseases of the sweet pea. Del. Agr. Exp. Sta. Bul. 93. 1914.
10. A Gloeosporium disease of the spice bush. Amer. Jour. Bot. **1**: 340–342. 1914.
11. The diseases of the sweet potato and their control. (Taubenhaus, J. J., and T. F. Manns). Del. Agr. Exp. Sta. Bul. 109. 1915.
12. The probable non-validity of the genera *Botryodiplodia*, *Diplodiella*, *Chaetodiplodia*, and *Lasiodiplodia*. Amer. Jour. Bot. **2**: 324–331. 1915.
13. Sweet potato diseases and their control (The John Lewis Russell Lecture). Trans. Mass. Hort. Soc. part **1**: 131–143. 1916.
14. Soil stain or scurf of the sweet potato. Jour. Agr. Res. [U. S.] **5**: 995–1002. 1916.
15. A contribution to our knowledge of silver scurf (*Spondylocladium atrovirens* Harz) of the white potato. Mem. New York Bot. Gard. **6**: 549–560. 1916.
16. On a sudden outbreak of cotton rust in Texas. Sci. (n.s.) **46**: 267–269. 1917.
17. The culture and diseases of the sweet pea. E. P. Dutton Co., New York. 1917.
18. Pox or pit (soilrot), of the sweet potato. Jour. Agr. Res. [U. S.] **13**: 437–450. 1918.
19. A text-book of mycology and plant pathology, by John W. Harshberger. (A review). Sci. (n.s.) **48**: 516–517. 1918.
20. Diseases of truck crops and their control. E. P. Dutton Co., New York. 1918.
21. Pink root of onions. Sci. (n.s) **49**: 217–218. 1919.
22. Field diseases of the sweet potato in Texas. Texas Agr. Exp. Sta. Bul. 249. 1919.
23. Storage and diseases of the sweet potato in Texas. Texas Agr. Exp. Sta. Bul. 250. 1919.
24. Recent studies on *Sclerotium rolfsii* Sacc. Jour. Agr. Res. [U. S.] **18**: 127–138. 1919.
25. Diseases of greenhouse crops and their control. E. P. Dutton Co., New York. 1920.
26. Wilts of the watermelon and related crops (Fusarium wilts of cucurbits). Texas Agr. Exp. Sta. Bul. 261. 1920.
27. Diseases of grains, sorghums, and millet, and their control in Texas. Texas Agr. Exp. Sta. Bul. 261. 1920.
28. A study of the black and yellow molds of ear corn. Texas Agr. Exp. Sta. Bul. 270. 1920.
29. On a peculiar disease of mulberry fruit. Nature-study Review. 282–285. (1921?)
30. Pink root disease of onions and its control in Texas. (Taubenhaus, J. J., and F. W. Mally). Texas Agr. Exp. Sta. Bul. 273. 1921.
31. Texas root rot of cotton and methods of its control. (Taubenhaus, J. J., and D. T. Killough). Texas Agr. Expt. Sta. Bul. 307. 1923.
32. The culture and diseases of the sweet potato. E. P. Dutton Co., New York. 1923.
33. The culture and diseases of the onion. (Taubenhaus, J. J., and Fred W. Mally). E. P. Dutton Co., N. Y. 1924.
34. Studies of a new Fusarium wilt of spinach in Texas. Texas Agr. Exp. Sta. Bul. 343. 1926.
35. Influence of moisture and temperature on cotton root rot. (Taubenhaus, J. J., and B. F. Dana). Texas Agr. Exp. Sta. Bul. 386. 1928.
36. Relation of cotton root rot and Fusarium wilt to the acidity and alkalinity of the soil. (Taubenhaus, J. J., Walter N. Ezekiel, and D. T. Killough). Texas Agr. Exp. Sta. Bul. 389. 1928.
37. Plants susceptible or resistant to cotton root rot and their relation to control. (Taubenhaus, J. J., B. F. Dana, and S. E. Wolff). Texas Agr. Exp. Sta. Bul. 393. 1929.
38. A method of inoculation for Phymatotrichum root rot investigations. (Taubenhaus, J. J., B. F. Dana, W. N. Ezekiel, W. J. Bach, and J. P. Lusk). Phytopath. **19**: 167–170. 1929.
39. A new cotton wilt. (Taubenhaus, J. J., W. N. Ezekiel, and H. E. Rea). Phytopath. **19**: 171–173. 1929.

40. Airplane photography in the study of cotton root rot. (Taubenhaus, J. J., Walter N. Ezekiel, and C. B. Neblette). Phytopath. **19**: 1025–1029. 1929.
41. Recent studies on Phymatotrichum root-rot. (Taubenhaus, J. J. and Walter N. Ezekiel). Amer. Jour. Bot. **17**: 554–571. 1930.
42. Studies on the overwintering of Phymatotrichum root rot. (Taubenhaus, J. J., and Walter N. Ezekiel). Phytopath. **20**: 761–785. 1930.
43. Soil-reaction effects on Phymatotrichum root rot. (Ezekiel, Walter N., J. J. Taubenhaus, and E. C. Carlyle). Phytopath. **20**: 803–815. 1930.
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(Several further papers on which Dr. Taubenhaus' name will appear are to be prepared from work completed or partly completed before his death).

STUDIES ON THE NUTRITION OF FUNGI

I. THIAMIN, ITS CONSTITUENTS, AND THE SOURCE OF NITROGEN[1]

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Fungi other than obligate parasites may be divided into 2 groups: those that manufacture their own auxithals (growth-promoting substances), and those that must secure these from extraneous sources. The first group grows readily on media composed of the essential inorganic salts and of dextrose, while the second group fails to grow on such media, regardless of time or environment, but must have, in addition, other substances. The identification of such substances constitutes the purpose of this series of papers.

Yeast and yeast extract contain auxithals for bacteria, fungi, higher plants, and animals. In addition to some of the known vitamins, of amino acids, other organic nitrogenous compounds, organic acids, carbohydrates, fats, and mineral salts, yeast contains a number of chemically unidentified, but physiologically active, "factors" that have been studied by animal physiologists; but it is not yet known if these factors are involved in the growth of fungi. The reader is referred to Lewis (11), Lecoq (9), and Von Euler (4) for a comprehensive review of these "factors," as well as of vitamins.

Outstanding among the known compounds of the yeast are the members of the Vitamin B complex and amino acids. The inorganic constituents are not considered here because the ash of yeast has been found to be without auxithallic properties. The carbohydrates also are excluded because all of our test organisms are capable of utilizing dextrose, and the addition to the medium of the carbohydrates extracted from the yeast has failed to show any activity. Other substances known to be present in yeast, such as succinic acid, uracil, glutathione, and ether-soluble materials, were tested and found ineffective. Nor did nicotinic and pimelic acids show any auxithallic property for our fungi, although Knight (8) found nicotinic acid to be essential for *Staphylococcus aureus*, and Mueller (14) reported that pimelic acid in minute amounts stimulated the diphtheria bacillus. Pantothenic acid, with or without inositol, proved inactive and heteroauxin failed to show any stimulation. This narrowed the field down to the source of nitrogen and to accessory growth substances, particularly thiamin.

The source of nitrogen is an important limiting factor in the nutrition of fungi, and the use of the "wrong" kind of nitrogen compound may often lead the investigator astray. For a comprehensive review of nitrogen assimilation, the reader is referred to Robbins (17). But, even the "right"

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kind of nitrogen, or combinations of nitrogenous compounds, often may be ineffective without the presence of thiamin.

Students of fungi did not devote much attention to the effect of vitamins until recently. Lepeschkin (10) found that 0.001 per cent of vitamin B (the different fractions of this vitamin complex were not known then), added to a culture solution with ammonium sulphate as the only source of nitrogen, caused a more rapid development of *Saccharomyces cerevisiae* and *Penicillium glaucum*. This stimulation was observed only during the earlier stages of growth; eventually, no difference could be detected between the controls and the treated cultures. In this connection it is interesting to note that Richards (16) arrived at a similar conclusion concerning the effect of pantothenic acid on yeast. Williams and Saunders (34) report that thiamin exerted very little effect on many strains of yeast, but "Old Process" yeast was stimulated by doses of 0.008 gamma and upwards. It should be stressed here that a substance that does not actually induce growth, but merely brings about a temporary stimulation, cannot be considered as a vital factor in the metabolism of fungi but merely as a stimulant; it seems, however, that thiamin, unlike most stimulants, also acts as a vital accessory factor in the nutrition of some fungi, as can be seen by the work of Schopfer (23), who showed that even such a minute dose as 0.0005 gamma per ml. of medium induced growth in *Phycomyces blakesleeanus*. In the absence of this vitamin, there was only a slight and submerged growth, whereas, in its presence, aerial mycelium, sporangiophores, as well as zygospores, developed very readily. Some 13 different amino acids, used separately or combined, failed to induce growth in this fungus. Pure riboflavin (vitamin B_2) was without any effect. Burgeff (3) confirmed and extended Schopfer's findings. Schopfer (24) extended his investigations to other fungi and found that *Absidia ramosa, Parasitella simplex, Mucor ramannianus, Dicranophora fulva, Chaetocladium brefeldii, Choanephora cucurbitarum, C. persicaria,* and *Pilaira anomala* also were induced to grow in synthetic media under the influence of thiamin. However, some other fungi, such as species of *Rhizopus,* failed to respond and some were even inhibited by the vitamin. Searching for growth factors other than thiamin, Schopfer (25) tested urine, Aspergillus extract (26), and wheat germ (27). He found that the urine factor that influenced the growth of *Phycomyces blakesleeanus* was neither thiamin nor auxin. The Aspergillus extract exerted the same influence upon the test fungus as thiamin. From wheat germ he separated 2 factors: while the nontreated wheat-germ extract induced growth in both *P. blakesleeanus* and *Rhizopus suinus,* the filtrate resulting from the animal-charcoal treatment proved inactive for *Phycomyces* and active for *Rhizopus,* while the eluate was active for *Phycomyces* and inactive for *Rhizopus.* The factors responsible for the growth of *Phycomyces* are believed by him to be disintegration products of thiamin, ineffective for animals but effective for the fungus. Kögl and Fries (6) noted that thiamin was the growth factor required by *Phycomyces blakesleeanus, Phytophthora cactorum, Nectria coccinia, Sclerotinia cinerea,*

Polyporus adustus, P. abietinus and *Tricholoma nudum.* The vitamin proved active also for *Fomes pinicola, Trametes cinnabarina,* and *T. serialis,* although malt extract and yeast extract proved more beneficial. When thiamin, inositol, and biotin (7) were combined, *Nematospora gossypii* (*Ashbya gossypii*) showed the greatest activity; slight growth resulted when these 3 constituents were used separately; biotin and inositol proved active but not so effective as when thiamin was added. Biotin, inositol, and thiamin induced the greatest growth in *Lophodermium pinastri,* biotin and thiamin proved less active, whereas, used separately, they failed to induce any growth.

For a review of the effect of thiamin on microorganisms and on higher plants the reader is referred to Bonner (2).

THE EFFECT OF CRYSTALLINE THIAMIN

At first, some 25 representative fungi were used as test organisms. These do not make an appreciable growth on a medium consisting of 1 g. of ammonium nitrate, 0.5 g. each of dihydrogen potassium phosphate and magnesium sulphate, 5 g. of dextrose, 20 g. of Bacto agar, and 1,000 ml. of distilled water. The addition of 1 g. of Bacto yeast extract to this medium, however, enabled all the organisms to grow. When a minute quantity (1 part per 20 million) of either synthetic or natural thiamin was used instead of yeast extract, 10 fungi were induced to grow. When the medium was modified by substituting a mixture of amino acids[2] for ammonium nitrate, and without the presence of thiamin, only one organism, *Basidiobolus ranarum,* grew readily, the remaining 24 continuing to make slight or no growth. The addition of thiamin to this modified medium enabled 14 organisms to make a rich growth. The results are listed in table 1. All nutrient media used throughout this work were adjusted to pH 5.5 before tubing.

Obviously, yeast extract contains more than one auxithal because all 25 fungi grow in its presence, while only 14 of them grow in presence of thiamin. The thiamin content of Bacto yeast extract is given by the manufacturers as 5 Sherman units per gram; too small a quantity to support a maximum growth. *Coprinus lagopus, Phytophthora erythroseptica, Pythiomorpha oryzae,* and *Pythium polymastum* failed to show a luxuriant growth in the presence of 0.1 per cent yeast extract, but attained it when the mixture of amino acids and thiamin were added to the synthetic medium. In so far as the performance of the 14 organisms listed in the last column is concerned, a suitable source of nitrogen and thiamin are the substances that control growth. One additional organism, *Basidiobolus ranarum,* grows readily without the vitamin, provided that the source of nitrogen is suitable. This leaves 10 fungi that require something other than thiamin or any of the foregoing amino acids for growth.

Riboflavin is the only other member of the vitamin B complex available

[2] *d*-arginine, *d*-glutamic acid, *l*-aspartic acid in 2 parts each; *dl-a*-alanine and glycine 1 part each. 1 gram of this mixture was used in each 1,000 ml. of medium.

TABLE 1.—*The effect of crystalline thiamin on the growth of fungi*

Synthetic medium	Synthetic medium plus yeast extract 1000 ppm.	Synthetic medium plus thiamin 0.05 ppm.	Synthetic medium with amino acid mixture (0.1%) in place of ammonium nitrate; no thiamin	Synthetic medium with amino acid mixture and thiamin 0.05 ppm.
None grew	Good growth *Allomyces javanicus* *Ascobolus viridulus* *Ashbya gossypii* *Basidiobolus ranarum* *Blakesleea trispora* *Ceratostomella multi-annulata* *Chaetocladium brefeldii* *Collybia tuberosa* *Dipodascus uninucleatus* *Lentinus tigrinus* *Nyctalis asterophora* *Ophiobolus oryzinus* *Pleurotus corticatus* *Phycomyces nitens* *Pilaira moreaui* *Pythiomorpha gonapodioides* *Pythium oligandrum* *Saprolegnia parasitica* *Sordaria fimicola* *Sporormia intermedii* *Thraustotheca clavata* Fair growth *Coprinus lagopus* *Pythiomorpha oryzae* *Phytophthora erythroseptica* *Pythium polymastum*	Good growth *Blakesleea trispora* *Collybia tuberosa* *Lentinus tigrinus* *Phycomyces nitens* *Phytophthora erythroseptica* *Pythimorpha gonapodioides* *Pythimorpha oryzae* *Pythium polymastum* Fair growth *Chaetocladium brefeldii* *Pythium oligandrum*	Good growth *Basidiobolus ranarum*	Good growth *Blakesleea trispora* *Collybia tuberosa* *Coprinus lagopus* *Chaetocladium brefeldii* *Lentinus tigrinus* *Nyctalis asterophora* *Phycomyces nitens* *Pilaira moreaui* *Phytophthora erythroseptica* *Pythiomorpha gonapodioides* *Pythiomorpha oryzae* *Pleurotus corticatus* *Pythium oligandrum* *Pythium polymastum*

commercially in the form of pure crystals. Added to the synthetic medium (1 ppm.) with ammonium nitrate as source of nitrogen, it failed to endow the medium with any auxithallic property; substitution of the amino acid mixture for ammonium nitrate proved only a little better, the stimulation being so slight as to be negligible.

A comparison of the third and last columns of table 1 shows that there might be a close relation between the activity of thiamin and the source of nitrogen. In order to demonstrate this more conclusively, 4 additional nitrogen compounds were used separately in place of the ammonium nitrate of the synthetic medium. In this and in all subsequent cases the amount of the nitrogen compounds used was 0.1 per cent.

The medium containing potassium cyanide was adjusted to pH 7 in order to prevent the production of hydrogen cyanide.

TABLE 2.—*The effect of different sources of nitrogen and thiamin upon growth*

Synthetic medium with sodium nitrate in place of ammonium nitrate and 0.05 ppm. thiamin	Sodium nitrite as source of nitrogen and 0.05 ppm. thiamin	Urea as source of nitrogen and 0.05 ppm. thiamin	Potassium cyanide as source of nitrogen and 0.05 ppm. thiamin
Good growth	Fair growth	Good growth	Good growth
Blakesleea trispora	*Blakesleea trispora*	*Coprinus lagopus*	*Lentinus tigrinus*
Collybia tuberosa		*Lentinus tigrinus*	*Pilaira moreaui*
Lentinus tigrinus		*Nyctalis asterophora*	*Pythium oligandrum*
Pythiomorpha gonapodioides		*Phycomyces nitens*	
		Pilaira moreaui	
Fair growth			Fair growth
Chaetocladium brefeldii		Fair growth	*Blakesleea trispora*
Phycomyces nitens		*Blakesleea trispora*	*Collybia tuberosa*
Pythiomorpha oryzae		*Phytophthora erythroseptica*	*Coprinus lagopus*
Pythium polymostum		*Pythiomorpha oryzae*	*Nyctalis asterophora*
			Phycomyces nitens

Table 2 supports the fact that thiamin is inactive unless the proper source of nitrogen is supplied. Sodium nitrate plus the vitamin induced growth in fewer organisms than ammonium nitrate because ammonium nitrogen can be utilized by more organisms than nitrate nitrogen. Sodium nitrite proved the poorest source of nitrogen; and, despite the presence of thiamin, only one organism, *Blakesleea trispora,* made a fair growth. It is doubtful if this fungus utilized nitrite nitrogen; the trace of nitrogen present in agar-agar was probably its only source of nitrogen. Urea was slightly more favorable than either sodium nitrate or potassium cyanide.

Agar-agar is freely used when solid media are desired; yet, it is a complex substance of unknown constitution. In order to determine the possible nutritional effect of agar-agar upon our organisms, 2 series of liquid cultures were prepared: the first series contained ammonium nitrate as the only source of nitrogen, while the second series received the amino acid mixture. Thiamin was added to both (0.05 ppm.). All 14 of the thiamin-responding organisms grew readily in the second series of solutions, while in the first series no or

only a slight growth was made by *Chaetocladium brefeldii, Collybia tuberosa, Pythiomorpha gonapodioides,* and *Pythium oligandrum.* Apparently, these fungi are unable to utilize ammonium nitrate without the presence of agar-agar. When nitrogen was left out of the medium, but the usual amount of the agar plus thiamin added, a fair growth was made by the foregoing as well as other organisms; but when ammonium nitrate was included, a rich growth followed. This means that some substance present in the agar enabled the fungi to utilize ammonium nitrate. While the Lassaigne test showed only a faint trace of nitrogen in agar-agar, we feel safe in assuming that this small amount of organic nitrogen was sufficient to give the mycelium the necessary start in the utilization of ammonium nitrate.

Since agar-agar merely duplicates, to a slight degree, the effect of the amino acids in the medium, the interpretation of data herein tabulated should not be complicated by the presence of agar.

THE EFFECT OF AMINO ACIDS AND THIAMIN

Some 24 individual amino acids were used singly and in several combinations. It was found that only 2 organisms, *Basidiobolus ranarum,* as shown before, and *Saprolegnia parasitica,* made good growth without the presence of thiamin. *Basidiobolus ranarum* made excellent growth in the presence of 0.1 per cent *d*-arginine and fair growth in the presence of any one of the following: *l*-aspartic acid, *d*-glutamic acid, glycine, *dl-a*-alanine, *dl*-phenylalanine, *l*-histidine, *dl*-valine, *dl*-leucine, and *dl*-serine. *Saprolegnia parasitica* grew only in the presence of either *l*-cystine or *dl*-leucine.

The remaining 23 fungi did not make an appreciable response to amino acids alone, although some were slightly stimulated. We are inclined to think that this stimulation was caused not by the amino acids themselves, but by the minute quantities of impurities present in them. A substance may be chemically pure, but biologically impure, because microorganisms are much more sensitive than chemical tests. Different lots of the same amino acid purchased from different manufacturers, and even the age of a given amino acid sold by the same firm, seemed to influence growth-stimulating properties. This is particularly applicable to the "natural" amino acids.

Combinations in 2's, 3's and 4's, as well as a mixture of all 24 of the amino acids, failed to show a marked advantage over the use of a single one in so far as the individual response of any one organism was concerned. However, where several organisms are to be cultured on a common medium, a combination of the more favorable amino acids will prove advantageous because, whereas one fungus attains its maximum growth in the presence of arginine, another does so in the presence of *dl-a*-alanine, or aspartic acid, etc. In case of fungi with a limited range of amino acid preference, such as *Saprolegnia parasitica,* the addition of either *l*-cystine or leucine to the mixture of amino acids will effectively extend the general usefulness of the medium.

In view of the response made by *Saprolegnia parasitica* to *l*-cystine, it was believed that some other organisms, which failed to grow on the synthetic

medium with the group of amino acids as source of nitrogen, and failed to respond to thiamin, probably would grow if the medium contained *l*-cystine. This proved to be the case, and *Saprolegnia mixta, Achlya conspicus, Isoachlia monilifera,* and *Aphanomyces camptostylus* made a rich growth.

It might be of interest to note the findings of other investigators concerning the effect of amino acids. Fildes and Richardson (5) have shown that tryptophane, leucine, phenylalanine, tyrosine, and arginine are indispensable for the growth of *Clostridium sporogenes,* while histidine, cystine, methionine, and valine are highly important and possibly indispensable. Anderson and Emmart (1) found that glycine, *l*-leucine, *l*-tyrosine, and *l*-aspartic acid did not produce stimulation of metabolism in *Fusarium oxysporum,* but *l*-aspartic acid and glycine were utilized by the fungus for the production of carbon dioxide, whereas leucine and tyrosine retarded the production of this gas. According to Tatum, Peterson, and Fred (32) *l*-asparagine is the factor in potato extract that stimulates butyl alcohol formation by certain butyric acid bacteria, and Tatum, Wood, and Peterson (33) have shown that the ammonium nitrogen and the asparagine of Neuberg precipitate fraction of potato extract stimulated a number of propionic acid bacteria. Mosher, Saunders, Kingery, and Williams (12) tested the effect of a number of amino acids upon *Trichophyton interdigitale* and found that no single amino acid was indispensable. Some proved to be very helpful, others less necessary, while still other amino acids had no specific effect. They found a varied assortment of amino acids to be superior to any group of 3 or 4; yet, given an adequate assortment of amino acids, the fungus would not grow unless supplied with at least one of the following: pantothenic acid, inositol, thiamin, or crude lactoflavin. These workers state that leucine proved the most important individual amino acid, with aspartic acid and *B*-hydroxy-alpha-amino butyric acid next in importance. Nielson (15) used 32 different amino acids in his work on nitrogen assimilation by *Saccharomyces cerevisiae* and observed a 99 per cent of assimilation of the following amino acids by the yeast: glycine, *dl*-aspartic acid, *l*-aspartic acid, and asparagine; 92–97 per cent assimilation of nitrogen was observed in case of *d-alanine, l*-tyrosine, *l*-leucine, *d-iso*-leucine, *d*-asparagine, and *dl*-glutamic acid. The yeast could not utilize *dl*-diaminopimelic acid, and only 1 per cent assimilation was noted in case of *d*-lysine and *dl*-diaminoadipic acid. All others varied from 2 to 81 per cent. Schopfer (24) states that in the presence of thiamin and with arginine taking the place of asparagine in the nutrient medium, *Phycomyces blakesleeanus* produced excellent growth, while tyrosine, lysine, and ornithine proved ineffective; serine and *dl*-proline were fair in this respect, and *d*-alanine, *l*-leucine, *d*-leucine, glumatic acid, hydroxyproline, and glycyl-glycine varied from slight to poor.

It is a well-known fact that different organisms do not always respond alike to the same food substances. Nor can we expect all fungi to utilze the same amino acids either with or without the aid of accessory growth factors. The table 3 illustrates this point. Twenty-four amino acids of 0.1

per cent concentration were tested separately, each replacing the ammonium nitrate of the synthetic medium. Crystalline thiamin at the rate of 1 part in 20 million was used as the accessory factor. This concentration of the vitamin is an arbitrary one; much greater dilutions gave equally good results and much higher concentrations failed to cause greater acceleration. Noticeable effects were seen, even in such a great dilution as one part of the vitamin in ten billion parts of the medium.

All results shown in table 2 are approximate; the exact relation of each organism to each of the amino acids tested can be determined only by the dry weight of the mycelium grown in nutrient solutions. Such a procedure probably would alter somewhat the order of availability of the amino acids, but the results would hardly justify the enormous amount of work involved.

The table 3 shows that every one of the 14 fungi is capable of making a good growth if given only one favorable amino acid as the source of nitrogen, and crystalline thiamin as the accessory factor. This seems not to accord with the results of some other workers who find that a given test organism requires more than one amino acid for best growth. The question is whether certain organisms utilize the amino acids *per se*, being incapable of synthesizing their own, or whether they must first break down the amino acids into more easily available forms and then proceed to build their own amino acids. If we accept the first theory, we shall be forced to admit that certain fungi can synthesize some of their amino acids, but must secure others from extraneous sources because proteins result from the combination of a number of amino acids instead of a few. As shown in table 1, 10 organisms can grow well in a synthetic medium with ammonium nitrate as the only source of nitrogen and thiamin as the accessory factor. Obviously, all 10 of these fungi are capable of synthesizing their own amino acids from the ammonium nitrate. Four additional organisms made a very good growth when the ammonium nitrate of the medium was substituted with a suitable amino acid. Since one amino acid does not make a protein, and since no organism used in this work has grown better on a combination of amino acids than on a single one, it is concluded that all 14 of the organisms manufacture their own amino acids. Nitrogen metabolism in these fungi depends on 3 factors: a suitable source of nitrogen, thiamin, and the enzymes of the organisms. Presumably the vitamin acts as a catalyzer; being effective in extremely minute quantities, the likelihood of its utilization as food is remote. This catalytic action probably occurs within the protoplasm in the presence of endoenzymes. Unless combined with some of the end products and rendered inactive, the vitamin should be present in the growing mycelium. Schopfer and Jung (30) were unable to obtain a positive test for thiamin when they fed to the experimental animals the mycelium of *Phycomyces blakesleeanus* grown in a medium containing the vitamin. This suggests one of the following possibilities: the vitamin was destroyed or reduced to its simpler components; it was combined with certain metabolic by-products and rendered inactive, or the mycelium did not contain enough thiamin to

TABLE 3.—*Amino acids as source of nitrogen with thiamin as accessory factor*

Organisms	The amino acids that were utilized by the fungi with the aid of thiamin
Blakesleea trispora	*d*-arginine, *l*-aspartic acid, *d*-glutamic acid, glycine, *dl-a*-alanine, *dl-a*-amino-caproic acid, *dl-a*-amino-*n*-butyric acid, *dl*-valine, *l*-proline, *dl*-leucine, *dl*-serine. Utilized, but not so well: *dl-iso*-leucine, *dl-a*-amino-*n*-caprylic acid, β-alanine, *l*-tyrosine.
Chaetocladium brefeldii	Glycine, *dl*-alanine. Utilized, but not so well: *d*-arginine, *dl-iso*-leucine.
Collybia tuberosa	*d*-arginine, *l*-aspartic acid. Utilized, but not so well: *dl*-serine.
Coprinus lagopus	*l*-aspartic acid, *d*-glutamic acid. Utilized, but not so well: *l*-tryptophane, *dl-iso*-leucine, *dl-a*-amino-caprylic acid.
Lentinus tigrinus	*d*-arginine, *l*-aspartic acid, *d*-glutamic acid, glycine, *dl-a*-alanine, *dl*-phenyl-alanine, *dl-iso*-leucine, *l*-cystine, *dl*-valine, *dl*-serine. Utilized, but not so well: *l*-tryptophane, *dl-a*-amino-*n*-caproic acid, *dl-a*-amino-caprylic acid, *dl-a*-amino-*n*-butyric acid, *l*-proline, *dl*-leucine.
Nyctalis asterophora	*d*-arginine, *d*-glutamic acid, glycine. Utilized, but not so well: *l*-aspartic acid, *dl-a*-alanine, *l*-tyrosine, *dl*-serine.
Phycomyces nitens	*d*-arginine, glycine, *dl-a*-alanine. Utilized, but not so well: *l*-aspartic acid, *d*-glutamic acid, *dl-iso*-leucine, *dl-a*-amino-caprylic acid, *l*-histidine, *dl*-serine.
Pilaira moreaui	Glycine, *dl*-alanine, *dl-iso*-leucine. Utilized, but not so well: *d*-arginine, *l*-aspartic acid, *d*-glutamic acid, β-alanine, *dl-a*-amino-caprylic acid, *l*-histidine, *l*-tyrosine, *dl*-leucine, *dl*-serine.
Phytophthora erythroseptica	*d*-arginine, *l*-aspartic acid, *d*-glutamic acid, glycine, *dl-a*-alanine, *l*-histidine, *l*-proline, *dl*-serine. Utilized, but not so well: *iso*-leucine, *l*-tryptophane, *dl*-valine.
Pleurotus corticatus	*l*-aspartic acid, *d*-glutamic acid. Utilized, but not so well: *d*-arginine, *dl-a*-alanine, *dl-iso*-leucine.
Pythiomorpha gonapodioides	*d*-arginine, *l*-aspartic acid, *d*-glutamic acid, glycine, *dl-a*-alanine, *dl-iso*-leucine, β-alanine, *l*-histidine, *l*-hydroxyproline, *l*-proline, *l*-tyrosine, *dl*-serine. Utilized, but not so well: *l*-tryptophane, *dl-a*-amino-*n*-butyric acid, *dl*-valine, *dl*-leucine.
Pythiomorpha oryzae	*d*-arginine, *l*-aspartic acid, *d*-glutamic acid, glycine, *l*-histidine, *l*-proline. Utilized, but not so well: *dl-a*-alanine, *l*-tryptophane, *l*-hydroxyproline, *dl*-valine, *l*-tyrosine, *dl*-serine.
Pythium oligandrum	*d*-arginine, *l*-aspartic acid, *d*-glutamic acid, glycine, *dl-a*-alanine, *dl-iso*-leucine, *l*-proline. Utilized, but not so well: β-alanine, *dl-a*-amino-*n*-butyric acid, *dl*-valine, *dl*-serine.
Pythium polymastum	*d*-arginine, *l*-aspartic acid, *d*-glutamic acid, *dl-a*-alanine, β-alamine, *l*-proline, *dl*-serine. Utilized, but not so well: glycine, *l*-histidine, *l*-hydroxyproline, *dl*-leucine.

supply the needs of the experimental animals. As fungi respond to much more minute doses of thiamin than do animals, an optimum dose for a fungus may still fall below the minimum for animals.

We used the following fungi to demonstrate whether or not a given organism is capable of manufacturing thiamin:

Cunninghamella blakesleeana grows very well on synthetic media containing neither thiamin nor constituents of thiamin. It was grown in 2 series of solutions, the first consisting of our synthetic medium with ammonium nitrate as the only source of nitrogen, and the second, containing our mixture of amino acids as the source of nitrogen.

Pythiomorpha gonapodioides was grown in the synthetic medium with the amino acids as source of nitrogen plus a mixture of the 2 intermediates of thiamin (2-methyl-5-ethoxymethyl-6-amino pyrimidine and 4-methyl-5-β-hydroxyethyl thiazole). Another series consisted of the same solution but containing only pyrimidine.

Phycomyces nitens was grown in the nutrient solution containing pyrimidine and thiazole.

Blakesleea trispora was grown in the synthetic solution containing amino acids and pyrimidine. The concentration of pyrimidine and thiazole was the same in all cases, 0.05 ppm. Each flask contained 200 ml. of the medium.

After a growth of 2 weeks in diffused light and at room temperature, the mycelium was removed, thoroughly washed, ground, added to the nutrient solution with the amino acids as source of nitrogen, heated in the autoclave for 5 minutes at 5 pounds' pressure, filtered, sterilized, and inoculated with: 1. *Pythiomorpha gonapodioides,* which grows readily in the synthetic solution containing pyrimidine only. 2. *Pilaira moreaui,* which requires both constituents of thiamin. 3. *Phycomyces nitens,* which has the same requirements as *Pilaira moreaui.* 4. *Phytophthora erythroseptica,* which cannot grow unless thiamin is present.

All 4 organisms grew very well in the presence of the mycelium extract of the 4 fungi listed above. Since *Phytophthora erythroseptica* grew very readily in all cases, we can safely assume that *Cunninghamella blakesleeana* synthesized its own thiamin from the synthetic solution; that *Pythiomorpha gonapodioides* can synthesize thiamin when furnished with pyrimidine, or from a mixture of pyrimidine and thiazole; that *Phycomyces nitens* prepares its own thiamin from a mixture of pyrimidine and thiazole; and that *Blakesleea trispora* can do the same with only pyrimidine in the solution. Some of the thiamin manufactured by the fungus is given off by the mycelium into the medium. In case of *Blakesleea trispora* the nutrient solution from which the mycelium was filtered off was tested and proved active for *Phytophthora erythroseptica.* Since the latter organism cannot grow in the presence of pyrimidine alone, it is a safe assumption that *Blakesleea trispora* excreted thiamin into the medium. The mycelium extract was more active than the medium.

Robbins and Kavanagh (31) have evidence showing that an organism

that grows well with an external supply of pyrimidine alone is capable of synthesizing thiazole, although it does not grow with thiazole alone. We have demonstrated that fungi synthesize their own thiamin from purely synthetic solutions, or from only one of the constituents of the thiamin, or from a mixture of pyrimidine and thiazole. If either constituent were capable of doing the work of thiamin, then why the elaborate process of thiamin synthesis? We agree with Robbins and Kavanagh that thiamin itself is the active agent in the metabolic activities of fungi and not its individual components.

THE EFFECT OF THE TWO INTERMEDIATES OF THIAMIN

Vitamin B_1 is a complex compound formed synthetically from 4-methyl-5-β-hydroxyethyl thiazole and 2-methyl-5-bromomethyl-6-amino pyrimidine. The question that naturally presents itself is whether these 2 intermediates are capable of replacing thiamin in nutrition tests before being linked. Schopfer (28) finds that the pyrimidine which he used (2-methyl-4(6)-amino-5-aminomethyl pyrimidine) increased the growth of 2 species of *Rhodotorula, R. rubra* and *R. flava,* whereas the thiazole was without any effect on *R. rubra* and slightly favorable to *R. flava.* Schultz, Atkin, and Frey (31) observed that Type A yeast was partly stimulated by either thiazole or pyrimidine and completely activated by the mixture of the two. Type B, on the other hand, was unaffected by thiazole, slightly inhibited by pyrimidine, and greatly inhibited by the mixture of the two. Schopfer and Jung[3] state that *Phycomyces blakesleeanus* is unable to grow in the presence of either thiazole or pyrimidine alone, but when these two are mixed, a good growth follows. Robbins and Kavanagh (18) obtained similar results with this organism, and Robbins (22) extended the work to numerous other organisms. Robbins and Kavanagh (21) confirmed Schopfer's results and extended their investigations to include a number of additional fungi. They demonstrated that, given the pyrimidine moiety, some were able to synthesize their own thiazole. Schopfer (29) states that *Absidia ramosa* and *Parasitella simplex* make only a slight growth in the control medium, or in the control to which thiazole is added, whereas in the presence of pyrimidine alone a fairly good growth follows. The maximum crop of mycelium, however, cannot develop without the presence of both intermediates or of thiamin. Müller and Schopfer (14) were unable to grow *Mucor ramannianus* without thiamin; pyrimidine failed to replace thiamin, but thiazole proved as effective as thiamin.

Through the courtesy of Merck & Co. we obtained the 2 intermediates of thiamin and used them at the same concentration as the vitamin (0.05 ppm.) with the amino acid mixture as the source of nitrogen. Table 4 gives the results.

When used alone, thiazole was unable to induce growth in any of the fungi, but, mixed with pyrimidine, it became a vital factor in the growth of *Chaetocladium brefeldii, Lentinus tigrinus, Pilaira moreaui,* and *Phycomyces*

[3] *Loc. cit.*

TABLE 4.—*The growth promoting qualities of the two intermediates of thiamin*

4-methyl-5-β-hydroxyethyl thiazole, 0.05 ppm. in nutrient medium	2-methyl-5-ethoxymethyl-6-amino pyrimidine, 0.05 ppm. in nutrient medium	0.05 ppm. each of the thiazole and pyrimidine in nutrient medium
No growth by any of the organisms	Good growth *Blakesleea trispora* *Collybia tuberosa* *Coprinus lagopus* *Nyctalis asterophora* *Pleurotus corticatus* *Pythiomorpha gonapodioides* *Pythium oligandrum* Poor to fair growth *Lentinus tigrinus* *Pilaira moreaui* No growth *Chaetocladium brefeldii* *Phycomyces nitens* *Phytophthora erythroseptica* *Pythiomorpha oryzae* *Pythium polymastum*	Good growth *Blakesleea trispora* *Chaetocladium brefeldii* *Collybia tuberosa* *Coprinus lagopus* *Lentinus tigrinus* *Nyctalis asterophora* *Phycomyces nitens* *Pilaira moreaui* *Pythiomorpha gonapodioides* *Pleurotus corticatus* *Pythium oligandrum* No growth *Phytophthora erythroseptica* *Pythiomorpha oryzae* *Pythium polymastum*

nitens. Only 3 organisms (*Phytophthora erythroseptica, Pythiomorpha oryzae,* and *Pythium polymastum*) remained unaffected by the pyrimidine, or by the pyrimidine and the thiazole mixed. These 3 fungi must have thiamin, which, apparently, is not broken up by the organisms into its 2 constituents before being utilized.

OTHER THIAZOLES AND PYRIMIDINES AS ACCESSORY FACTORS

After the synthesis of thiamin was accomplished, many analogues of vitamin B_1 were prepared and tested on experimental animals. Unfortunately, these are not generally available, and we know of only Robbins and Kavanagh (20) who have tested at least one of these analogues on fungi. Their experimental organism, *Phycomyces blakesleeanus*, failed to make any growth on it; upon the addition to the analogue of the pyrimidine fraction of vitamin B_1, a good growth followed. This probably was because the modification in the analogue was in the pyrimidine moiety, the thiazole remaining the same. In addition to the thiamin analogues, there have been many pyrimidines and thiazoles prepared and tested. Robbins and Kavanagh (20) have done the most extensive work along this line, using *Phycomyces blakesleeanus* as test organism. They tried some 37 various pyrimidines in conjunction with 4-methyl-5-β-hydroxyethyl thiazole and found that the pyrimidine compounds were active only when they possessed an amino group in position 6 and a mono-substituted methyl group in position 5. They (19) also tested 13 various thiazoles in conjunction with 2-methyl-5-bromomethyl-6-aminopyrimidine and 2-methyl-5-ethoxymethyl-6-amino pyrimidine, and found that activity for thiazoles depends upon their configuration; there must be a hydrogen in position 2 and a β-hydroxyethyl group in position 5; the hydroxyl must be free for greatest activity.

We tested 5 different pyrimidines; the test organisms were those of our fungi that responded to the pyrimidine nucleus of thiamin, or to pyrimidine and thiazole mixed. We confirm the conclusions of Robbins and Kavanagh[4] that the amino group in position 6 is necessary for activity. At a concentration of 0.05 ppm. we found no difference in activity with the following groups in position 5: ethoxymethyl, hydroxymethyl, and bromomethyl. When the amino group in position 6 was replaced by chlorine or oxygen, the activity was lost. We tested 2-methyl-5-hydroxymethyl-6-amino pyrimidine and found it to be equally effective as 2-methyl-5-bromomethyl-6-amino pyrimidine or 2-methyl-5-ethoxymethyl-6-amino pyrimidine. Thus the $-CH_2OH$ group may be added to the list of Robbins and Kavanagh ($-CH_2Br$, $-CH_2OC_2H_5$ and $-CH_2NH_2$) as being an effective substituant in position 5.

We also tested 4 different thiazoles and observed that ethyl-4-methyl-thiazole-5-carboxylate (H. Clarke) was able to replace 4-methyl-5-β-hydroxyethyl thiazole for some of the organisms that require both constituents of thiamin. We confirm Robbins and Kavanaugh that 4-methyl-5-β-ethoxyethyl thiazole picrate (H. Clarke) is less active for *Phycomyces blakesleeanus* than 4-methyl-5-β-hydroxyethyl thiazole, although no such decrease in activity was noted in case of the other organisms.

THE EFFECT OF PROLONGED AUTOCLAVING UPON THE ACCESSORY GROWTH FACTORS

It is a well-established fact that thiamin in alkaline solution (pH 10) is destroyed when autoclaved at 15 lb. for 5 hours. The nature of this destruction is not known; the vitamin may break down into simpler compounds or perhaps a molecular rearrangement takes place and destroys the prophylactic property of the vitamin. Since thiamin apparently does not always function the same way for fungi as it does for animals, it would be of interest to find what effect, if any, prolonged autoclaving of the vitamin would have upon the growth response of fungi.

Four solutions were prepared: the first contained yeast extract (1000 ppm.), to the second was added thiamin (0.05 ppm.), the third had pyrimidine, and the fourth thiazole and pyrimidine (0.05 ppm. each). The solutions were adjusted to pH 10 by means of sodium hydroxide and autoclaved continuously for 5 hours at 15 lb. The pH was then adjusted to 5.5 with dilute sulphuric acid, the nutrient constituents and agar were added, tubed, sterilized, and inoculated. Table 5 summarizes the results:

Only 4 out of 14 fungi failed to grow in the medium containing the autoclaved thiamin, the remaining organisms, apparently, are capable of utilizing the by-products that result from thiamin decomposition. Four organisms (4th column) failed to grow in the presence of the autoclaved pyrimidine and thiazole mixture, although in this instance failure to grow cannot be ascribed to autoclaving, as, with the exception of *Collybia tuberosa*, the other 3, *Pythium erythroseptica*, *Pythiomorpha orzae*, and *Pythium polymastum*, do not respond to a mixture of pyrimidine and thiazole. In case of *Phycomyces*

[4] *Loc. cit.*

TABLE 5.—*The effect of prolonged autoclaving upon the growth promoting properties of yeast, thiamin, and the two components of thiamin (5 hrs. 15 lbs. pH 10)*

Yeast extract 1000 ppm.	Thiamin 0.05 ppm.	2-methyl-5-ethoxy-metyl-6-amino pyrimidine, 0.05 ppm.	2-methyl-4-ethoxy-methyl-6-amino pyrimidine 0.05 ppm. plus 4-methyl-5-hydroxyethyl thiazole 0.05 ppm.
Good growth	Good growth	Good growth	Good growth
Blakesleea trispora	*Blakesleea trispora*	*Blakesleea trispora*	*Blakesleea trispora*
Chaetocladium brefeldii	*Coprinus lagopus*	*Coprinus lagopus*	*Coprinus lagopus*
Coprinus lagopus	*Lentinus tigrinus*	*Nyctalis asterophora*	*Lentinus tigrinus*
Lentinus tigrinus	*Nyctalis asterophora*	*Pythiomorpha gonapodioides*	*Nyctalis asterophora*
Nyctalis asterophora	*Pythiomorpha gonapodioides*	*Pythium oligandrum*	*Phycomyces nitens*
Phycomyces nitens	*Pleurotus corticatus*		*Pilaira moreaui*
Pilaira moreaui	*Pythium oligandrum*		*Pythiomorpha gonapodioides*
Pythiomorpha gonapodioides			*Pleurotus corticatus*
Pleurotus corticatus			*Pythium oligandrum*
Pythium oligandrum			
Fair growth	Fair growth	Fair growth	Fair growth
Pythium polymastum	*Chaetocladium brefeldii*	*Lentinus tigrinus*	*Chaetocladium brefeldii*
	Pilaira moreaui	*Pleurotus corticatus*	
	Pythium polymastum		
No growth	No growth	No growth	No growth
Collybia tuberosa	*Phycomyces nitens*	*Chaetocladium brefeldii*	*Collybia tuberosa*
Phytophthora erythroseptica	*Collybia tuberosa*	*Collybia tuberosa*	*Phytophthora erythroseptica*
Pythiomorpha oryzae	*Phytophthora erythroseptica*	*Pilaira moreaui*	*Pythiomorpha oryzae*
	Pythiomorpha oryzae	*Phycomyces nitens*	*Pythium polymastum*
		Phytophthora erythroseptica	
		Pythiomorpha oryzae	
		Pythium polymastum	

nitens the autoclaved mixture of pyrimidine and thiazole remained active, although autoclaved thiamin lost all activity. *Pythium polymastum,* which in previous experiments seemed to require thiamin, made a fair growth in the presence of autoclaved thiamin, as well as of yeast extract. This suggests two possibilities: either this organism is capable of utilizing some thiamin by-product or else it is able to dissociate into a form capable of using some of the intermediates. Dissociation is by no means uncommon, and 2 organisms in particular, *Chaetocladium brefeldii* and *Lentinus tigrinus,* have given some erratic responses that cannot be explained by any other theory.

FRACTIONATION OF YEAST EXTRACT

It has been seen that yeast extract is capable of supporting growth not only in organisms requiring thiamin or its moieties but also in those fungi that do not respond to thiamin. We endeavored to separate these 2 factors from the yeast extract. It should be stressed here that chemical methods of separation and concentration may often be very destructive. For instance, from 250 kg. of dried egg yolk, Kögl obtained only 1.1 mg. of biotin. He

estimates that over 98 per cent of the biotin was destroyed during the process of purification. Perhaps this is one reason why animal physiologists have purified so few of their "factors" found in yeast.

The yeast auxithals that induced growth in our test organisms are readily adsorbed on neutral norit from which they may be eluted by means of hot pyridine-water (2:1). The non-adsorbed fraction, consisting of 90 per cent of the original yeast extract proved entirely inactive. The eluate was dried in vacuo (20 mm.), dissolved in pyridine-water and precipitated by 10 volumes of 95 per cent ethanol. The precipitate, being inactive, was discarded. The filtrate was taken to dryness, dissolved in 90 per cent formic acid, and precipitated by 15 volumes of acetone. The inactive precipitate was discarded; the filtrate was taken to dryness by vacuum distillation, dissolved in 5 per cent sulphuric acid, and precipitated with 50 per cent phosphotungstic acid in 5 per cent sulphuric acid. The filtrate was inactive. The precipitate was regenerated with barium hydroxide and sulphuric acid and, after drying, extracted with hot absolute alcohol. The residue was discarded, the filtrate was "esterified" by adding 1 ml. of concentrated sulphuric acid and by refluxing for 2 hours. The sulphuric acid was removed with barium carbonate and the alcohol by distillation. The dry residue was then treated with boiling chloroform. The chloroform-soluble fraction (A) proved active for organisms requiring thiamin, while the chloroform-insoluble fraction (B) proved active for the organisms that failed to respond to thiamin.

Vacuum sublimation at 0.1 mm. and 150° C. of the material regenerated from the phosphotungstic precipitate yielded needle-like, double-refractive crystals that proved active for the organisms requiring thiamin, but inactive for the other group. The unsublimed residue proved active for organisms that failed to respond to thiamin. A more clear-cut separation was obtained by sublimation than by chloroform fractionation. There was, however, a greater destruction of fraction B because of the high temperature used in sublimation.

Fraction A may or may not be a mixture of thiazole and pyrimidine, but we feel safe in assuming that the needle-like crystals we have separated cannot be a mixture of these two. The greatest dilution in which these crystals show noticeable activity is 1 part in 5 million, whereas pyrimidine, or pyrimidine and thiazole in mixture, is highly active in much greater dilutions. It is possible that the crystals are inactive and that they carry the active principle as adsorbed impurity. Further work will clear this point.

Fraction B is bios-like in its action; it supports excellent growth of various strains of *Saccharomyces cerevisiae*. Possibly, it is related to biotin or contains biotin in addition to other auxithals. It has, however, about the same degree of activity as fraction A, which shows that its biotin content is rather small. (Pure biotin is active in 1 part per 250 billion.) We have fairly good evidence that this fraction contains biotin because *Ashbya gossypii* does not make any growth in the presence of fraction B in our nutrient medium nor in the presence of inositol, but, when inositol (100 ppm.) and fraction B (10

ppm.) were added together to the medium, a rich golden growth resulted. Kögl and Fries (6) obtained similar results with this organism when they used biotin and inositol, whereas no growth occurred when these two were tested separately.

SUMMARY

Twenty-five fungi used as test organisms failed to grow on a synthetic medium consisting of 1 g. ammonium nitrate, 0.5 g. each of dihydrogen potassium phosphate and magnesium sulphate, 5 g. of dextrose, 20 g. of agar, and 1,000 ml. of distilled water. When a minute quantity of crystalline thiamin was added to this medium (0.05 ppm.) ten of the fungi grew very well; but when a mixture of amino acids was substituted for ammonium nitrate, 4 additional organisms made a good growth.

Sodium nitrate, sodium nitrite, potassium cyanide, and urea were substituted for ammonium nitrate in the foregoing medium, with the vitamin as the accessory factor. Only 1 organism made some growth in the medium containing sodium nitrite. Sodium nitrate, potassium cyanide, and urea were not so favorable as ammonium nitrate.

Twenty-four amino acids were used singly or in combinations to replace the ammonium nitrate of the medium. In case of 2 organisms, *Basidiobolus ranarum* and *Saprolegnia parasitica,* amino acids proved to be the growth-inducing substances; the remaining 23 were unaffected. The addition of thiamin to the proper amino acids induced growth in 14 fungi. Nine organisms failed to respond to the amino acids plus thiamin.

The 2 intermediates of thiamin were tested, 4-methyl-5-β-hydroxyethyl thiazole was inactive when used alone; 2-methyl-5-ethoxymethyl-6-amino pyrimidine induced growth in 9 organisms; the 2 constituents together induced growth in 11 organisms; 3 fungi responded only to thiamin.

Five different pyrimidines and 4 different thiazoles were tested, confirming that the amino group in position 6 is necessary for the activity of pyrimidine and that there must be a hydrogen in position 2 and a β-hydroxyethyl group in position 5 for the activity of thiazole.

Solutions of thiamin, of pyrimidine, and of pyrimidine and thiazole together were adjusted to pH 10 and autoclaved for 5 hours at 15 lb., adjusted to pH 5.5, added to the nutrients and tested. Autoclaved thiamin induced growth in 10 organisms as against 14 that grew in media containing non-autoclaved thiamin. Some organisms that failed to grow in the presence of autoclaved thiamin grew when the autoclaved pyrimidine and thiazole mixture was added to the medium.

A method is described whereby yeast extract was fractionated to yield two components. The first of these induced growth in the organisms that responded to a mixture of thiazole and pyrimidine, while the other fraction proved active for the organisms that failed to respond to thiamin or its intermediates.

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ACQUIRED TOLERANCE TO CURLY TOP IN THE TOMATO[1]

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When young tomato plants under field conditions are inoculated in late spring with the curly-top virus, symptoms of the disease usually appear on infected plants in about 12 days. The leaves become rolled and the plant assumes a sulphur-yellow color. Growth is checked, flowers and buds drop, and the fruit colors prematurely. Plants that have already matured fruit, and those raised from cuttings of more mature plants are less easily infected, but when infection occurs the symptoms are the same. As a rule, the affected plant dies, but in some instances severely diseased plants have been observed to make new growth, which suggests at least a partial recovery from the disease.

The term "recovery" in the present sense means a process of regeneration in which relatively healthy shoots arise from the leaf axils. New, apparently healthy shoots sometimes arise from the lower nodes of severely diseased plants after the almost complete death of the top-growth. If a plant recovers sufficiently early in the season, and if the growth of the regenerated parts is more or less normal, the plant may yield mature fruit. The new growth is usually not quite normal, especially at the beginning. It may be feeble and yellowish, or fairly healthy, with only a slight yellow and purple tinge suggesting mild symptoms of curly top.

If recovery be slow and feeble a relapse may occur, resulting in death of the plant. The factors causing the relapse of tomato plants after they show recovery are not understood. A case is reported by Lesley[4] of a recovered

[1] Cooperative investigations between the University of California Citrus Experiment Station and the Division of Sugar Plant Investigations, Bureau of Plant Industry, U. S. Department of Agriculture. Paper No. 386, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

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[4] Lesley, J. W. The resistance of varieties and new dwarf races of tomato to curly top (western yellow blight or yellows). Hilgardia **6**: 27–44. 1931.

TABLE 1.—*Observed cases of recovery from curly-top symptoms by regeneration in tomato seedlings inoculated at Riverside, California, 1928–1937*

Races, hybrids, and commercial varieties	Number of plants				Percentage recovered
	Inoculated	Diseased	Recovered	Relapsed	
WILD RACES AND RACE HYBRIDS:					
Lycopersicum pimpinellifolium No. 497	10	10	5	2	50
" " F.P.I. 79532	10	9	5	0	56
" " F.P.I. 97850	20	19	19	0	100
No. 381–1	....	3[a]	1	0	33
No. 503	26	26	11	0	42
F_1; No. 573 × *L. pimpinellifolium* No. 548	10	10	1	0	10
F_1; No. 503 × " " F.P.I. 79532	24	13	10	0	83
F_1; No. 573 × " " F.P.I. 79532	23	11	0	0	0
F_1; No. 381–1 × " " No. 548	23	10	4	0	40
Guasave A	40	37	33	1	89
Guasave A, Recovered parents selfed	22	22	12	3	55
Guasave B	8	6	2	0	33
Ecuador	11	11	9	5	82
COMMERCIAL VARIETIES:					
Stone, Norton, Santa Clara, Marglobe	211	131	9	4	7

[a] These plants became infected naturally.

tomato plant that, following a heavy inoculation the same season, again developed severe curly-top symptoms. However, in most instances reinoculation of recovered plants has had no visible effect on the plants.

Under field conditions, plants that develop curly-top symptoms early in the season are probably less likely to recover than those developing symptoms later. In 1931 and 1933, at Riverside, among 53 plants that showed curly top early in the season, there was 15 per cent recovery, while of 20 plants that developed symptoms later in the season there was 25 per cent recovery.

Although recovery by regeneration appears to be influenced both by weather conditions and the stage of growth of plants at the time of appearance of symptoms, the ability to recover is a racial characteristic also. Some cases of recovery in tomato races and hybrids observed at Riverside from 1928 to 1937 are listed in table 1. In one wild race, Guasave A, from western Mexico, the proportion of plants that recovered was especially high, varying from 55 to 100 per cent in different years. In other races, including several commercial varieties not listed in table 1, hardly any plants recovered. A race from Ecuador was "high" in recovery but seemed subject to relapse.

A small number of F_1 plants of "high recovery" wild races, No. 503 × F.P.I. 79532 was high in recovery. F_1 hybrids of high-recovery race F.P.I. 79532 and another *pimpinellifolium* race No. 548 with No. 573, a wild race whose power of recovery is not known, were decidedly low in recovery. Seedlings from a recovered plant seem to have no greater ability to recover than seedlings from the ordinary stock seed of the race.

Clones derived from tomato plants that recovered from curly top by regeneration have an apparent resistance or tolerance not present in clones from disease-free, noninoculated plants. The results of inoculations of clones from both sources are compared in table 2.

In 1934, 7 plants from cuttings of hybrid C 263–1, which had recovered the previous year, were exposed to infection in the field by caging 10 viru-

TABLE 2.—*Effect of inoculation of tomato clones derived from plants that recovered from curly top and of clones from healthy, noninoculated plants*

Parentage of clones	Number of progeny plants	
	Inoculated	Diseased[a]
Clones from recovered parents:		
Hybrid C 263–1 (Wild)	7	0
No. 381–1 (Wild race) × *Lycopersicum pimpinellifolium* No. 548	23	4[b]
Guasave A (Wild)	34	0
Clones from healthy, noninoculated parents:		
Goffs (Wild)	8	6[c]
Selection 36.033.15 (Commercial)	19	13[c]

[a] Clones from recovered parents usually showed slight curly-top symptoms when inoculated.

[b] Symptoms on these plants became more pronounced following inoculations.

[c] These plants became severely diseased and all died.

liferous beet leaf hoppers on each plant. None of these plants developed curly-top symptoms any more pronounced than those shown at the time of reinoculation, whereas 43 per cent of the seedling plants of several races similarly treated developed severe symptoms.

An F_1 plant from 2 primitive races, No. 381–1 and No. 548, which showed recovery in 1933, was propagated by cuttings for 3 years. In 1934, 5 plants, and in 1935, 9 plants of this clone were inoculated in the field. In 1936, 9 plants were inoculated twice during the season. Four of the above 23 plants, at some time following the inoculations, developed more pronounced curly-top symptoms than they had shown at the time of inoculation, but it could not be determined if the increased severity resulted from the inoculations. Two of the 4 plants again recovered and produced a fair crop. The other 19 plants were not visibly affected by the inoculations. During 1935 and 1937, 34 plants from cuttings of recovered plants of Guasave A were reinoculated without any noticeable effect. On the other hand, 27 cuttings from healthy, previously noninoculated plants of 2 other races were inoculated and 19 developed extremely severe symptoms with no recovery.

In order to test for the presence of virus, nonviruliferous beet leaf hoppers were caged on portions of recovered tomato plants for 2 to 3 days and then transferred to healthy seedling beets of a variety susceptible to curly top. Tests were made on 21 tomato plants originating from cuttings from seedlings that recovered the previous year and virus was obtained from each. The percentages of leaf hoppers acquiring virus from the recovered tomato plants and the severity of symptoms produced on the test beets indicated that the tomato plants contained a rather high concentration of virus, and further, that its virulence was unchanged. However, further studies are necessary to determine definitely if any qualitative change of the virus occurs. Tests also were made of plants in the process of recovery. Virus was easily obtained from regenerated, almost symptomless shoots and from severely diseased portions of the same plants. On the other hand, healthy appearing clones taken from plants not experimentally inoculated and not showing curly-top symptoms during the seedling year were tested the following season, before they were inoculated, and were found not to contain virus. When inoculated, such clonal plants developed severe symptoms of curly top. Thus, it seems that the presence of virus in the regenerated plants is a prerequisite for acquired tolerance; at least, tolerance developed only in plants after they had been infected, and the virus remained in the recovered plants and apparently continued to multiply. Nothing is known of how recovered plants would react to reinoculation if they could be entirely freed of virus.

The phenomenon of recovery of tomato plants from curly top is similar to that of recovery of tobacco plants from the ring-spot disease first reported by Wingard,[6] and later studied extensively by Price.[5] In tomatoes, how-

[5] Price, W. C. Acquired immunity to ring-spot in Nicotiana. Contrib. from Boyce Thompson Inst. 4: 359–403. 1932.

[6] Wingard, S. A. Hosts and symptoms of ring spot, a virus disease of plants. Jour. Agr. Res. [U. S.] 37: 127–154. 1928.

ever, fewer of the plants recover and recovery seems not to be so complete as that reported by both Wingard and Price in their studies of tobacco ring spot.

Tomato plants of a recovered clone are relatively slow-growing and have other mild curly-top symptoms characteristic of regenerated shoots. In spite of this condition they may be fairly productive. In 1937, out of 18 plants from a recovered clone of Guasave A that survived a fairly heavy inoculation in addition to the rather high concentration of virus already present, 10 plants produced a good crop of fruit (Fig. 1) and the remainder

FIG. 1. Acquired tolerance in a tomato plant of a Guasave A clone derived from a seedling that recovered from curly top. The mature fruits are crimson and averaged 0.5 oz. in weight. (Photo. Oct. 11, 1937.)

a light crop, which matured about the normal time. All the plants were of less than normal size, even allowing for closeness of planting. Reinoculation of such plants in the summer had no noticeable effect on them. Clones from healthy plants of other races inoculated at the same time developed severe curly-top symptoms and many of them died; the surviving affected plants produced little or no fruit. In 1937, 19 seedlings of Guasave A were inoculated and all of them developed severe curly top. All of these seedling plants showed regeneration, but recovery was too late for the production of a crop that season.

In some areas of the western United States curly top is the limiting factor in tomato production. So far, attempts to select or develop resistant commercial varieties have largely failed. No race of tomato, either wild or cultivated, has been found that is highly resistant to initial infection with

curly-top virus. There is some difference in the reaction of certain races or varieties of tomato to curly top but none have shown sufficient resistance to make them of much practical value. Tomato plants affected with curly top do, however, sometimes recover, in part, at least, and acquire a tolerance to the virus. The tomato plants that acquired tolerance to curly top in these studies, with one possible exception, belonged to wild races. Recovery has been observed in cultivated varieties, but, since an intensive study of this problem was begun, sufficient material has not been available to permit conclusions regarding the association of acquired tolerance with recovery in cultivated varieties.

It may be possible to develop by hybridization a desirable tomato, high in recovery from and tolerant to curly top, and of sufficiently early maturity to enable recovered seedlings to produce a satisfactory crop during the first season's growth. However, if a clone of a larger-fruited variety than Guasave A can be obtained, with similar tolerance to curly top, it would be worth while to overwinter it and propagate it by cuttings for use in areas where curly top seriously limits tomato production.

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INHERITANCE OF RESISTANCE TO TOBACCO-MOSAIC DISEASE IN TOBACCO

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In 1914, Allard (1) found that the failure of *Nicotiana glutinosa* L. to show chlorosis after inoculation with tobacco-mosaic virus was shared by the hybrid *N. tabacum* L. × *N. glutinosa*. This hybrid has since been found to respond to infection with tobacco-mosaic virus, as *N. glutinosa* does, by the production of necrotic primary lesions (7, p. 992; 9). In these lesions the virus is localized except in young plants where systemic necrosis frequently occurs. Whether the necrosis is localized or systemic, ordinary contaminative contacts between leaves of infected and healthy individuals are ineffective in spreading the disease, in contrast to the high infectivity associated with the systemic chlorosis caused by infection with tobacco-mosaic virus in *N. tabacum*.

In preliminary experiments, designed to introduce into *Nicotiana tabacum* the necrotic type of response to infection characteristic of *N. glutinosa*, attempts were made to obtain seed from the first generation hybrid *N. tabacum* × *N. glutinosa*. Plants of this hybrid were grown continuously in greenhouse and garden, often in considerable numbers, for more than 3 years. During this period the hybrid proved consistently self-sterile and sterile to all tested pollens. Finally, because of the failure of repeated attempts to

cross it with varieties of *N. tabacum,* a fertile amphidiploid derived from this hybrid was obtained through the kindness of Dr. R. E. Clausen of the University of California. This amphidiploid species, *N. digluta* Clausen and Goodspeed, was found to resemble *N. glutinosa* in its response to infection with tobacco-mosaic virus. It was therefore used in further breeding experiments. The purpose of this paper is to report the segregation of disease types in successive generations.

EXPERIMENTS WITH DERIVATIVES OF NICOTIANA DIGLUTA

The species *Nicotiana digluta,* described by Clausen and Goodspeed (2) as a self-fertile amphidiploid originating from the hybrid *N. glutinosa* (n = 12) × *N. tabacum* (n = 24), has been studied intensively in the past, but not from the point of view of disease resistance. Derivatives of the form *N. digluta* × *N. tabacum* and (*N. digluta* × *N. tabacum*) × *N. tabacum* were produced and described by Clausen (3). Since they were not tested by inoculation, however, it is not known whether the necrotic type of response was retained in any individuals of this series of hybrids. In such repeated backcrosses, any characteristic dependent on a gene, or genes, introduced from the non-recurrent parent naturally would be eliminated unless specifically demonstrated and retained in each generation.

As a preliminary to the study of disease types in hybrid generations, 75 plants of *Nicotiana digluta* were tested by inoculation with tobacco-mosaic virus, applied by rubbing. They all produced necrotic local lesions resembling those of *N. glutinosa.* On maturing they proved self-fertile and also set seed readily when emasculated flowers were treated with pollen from *N. tabacum.*

In the first hybrid generation, *Nicotiana digluta* × *N. tabacum* var. Connecticut Broadleaf, 132 plants were grown and tested. They all responded to infection with tobacco-mosaic virus by production of necrotic primary lesions like those of *N. digluta* and *N. glutinosa.*

The F_1 plants were crossed with 3 varieties of *Nicotiana tabacum,* Connecticut Broadleaf, White Burley, and Samsoun tobacco. Segregation of the parental disease types occurred among the progeny. Thus (*N. digluta* × *N. tabacum* var. Connecticut Broadleaf) × *N. tabacum* var. Connecticut Broadleaf gave a ratio of 121 necrotic-type plants to 504 chlorotic-type plants. (*N. digluta* × *N. tabacum* var. Connecticut Broadleaf) × *N. tabacum* var. White Burley gave 272 necrotic-type to 269 chlorotic-type plants. (*N. digluta* × *N. tabacum* var. Connecticut Broadleaf) × *N. tabacum* var. Samsoun gave 310 necrotic-type to 357 chlorotic-type plants.

The results of tests of the first backcross generation, together with those of subsequent backcrossed and selfed generations, are shown in table 1.

Except among the derivatives of Connecticut Broadleaf tobacco, it will be seen from the table that in successive generations the ratios of necrotic-type to chlorotic-type plants, though at times deficient, tended to become the typical monohybrid 1:1 backcross and 3:1 selfed ratios indicative of the

TABLE 1.—*Ratios of necrotic-type to chlorotic-type plants among derivatives of Nicotiana digluta Clausen and Goodspeed*

F_1 hybrid and backeross generations[a]	Number of times with *N. tabacum* as parent[c]	Connecticut Broadleaf backeross line	Burley (Burley 16) backeross line	Samsoun backeross line
N. digluta × *N. tabacum* F_1 ...	2	132 : 0	.....	.....
B_1	3	121 : 504	272 : 269	310 : 357
B_2	4	17 : 247	99 : 148	93 : 171
B_3	5	23 : 286	123 : 120	96 : 149
B_4	6	63 : 185	156 : 115	120 : 135
Selfed generations[b]	**Number of times selfed**	**Connecticut Broadleaf selfed line**	**Burley selfed line**	**Samsoun selfed line**
F_2	1	310 : 144	325 : 136	324 : 110
F_3	2	271 : 182	328 : 121	399 : 0
F_4	3	123 : 79	347 : 128	303 : 0

[a] Beginning with the necrotic-type hybrid *N. digluta* × *N. tabacum* variety Connecticut Broadleaf, backcrosses were made to the three varieties of *N. tabacum*, Connecticut Broadleaf, Burley, and Samsoun, a necrotic-type hybrid of the preceding generation being used as ♀ parent, *N. tabacum* as ♂ parent in each case.

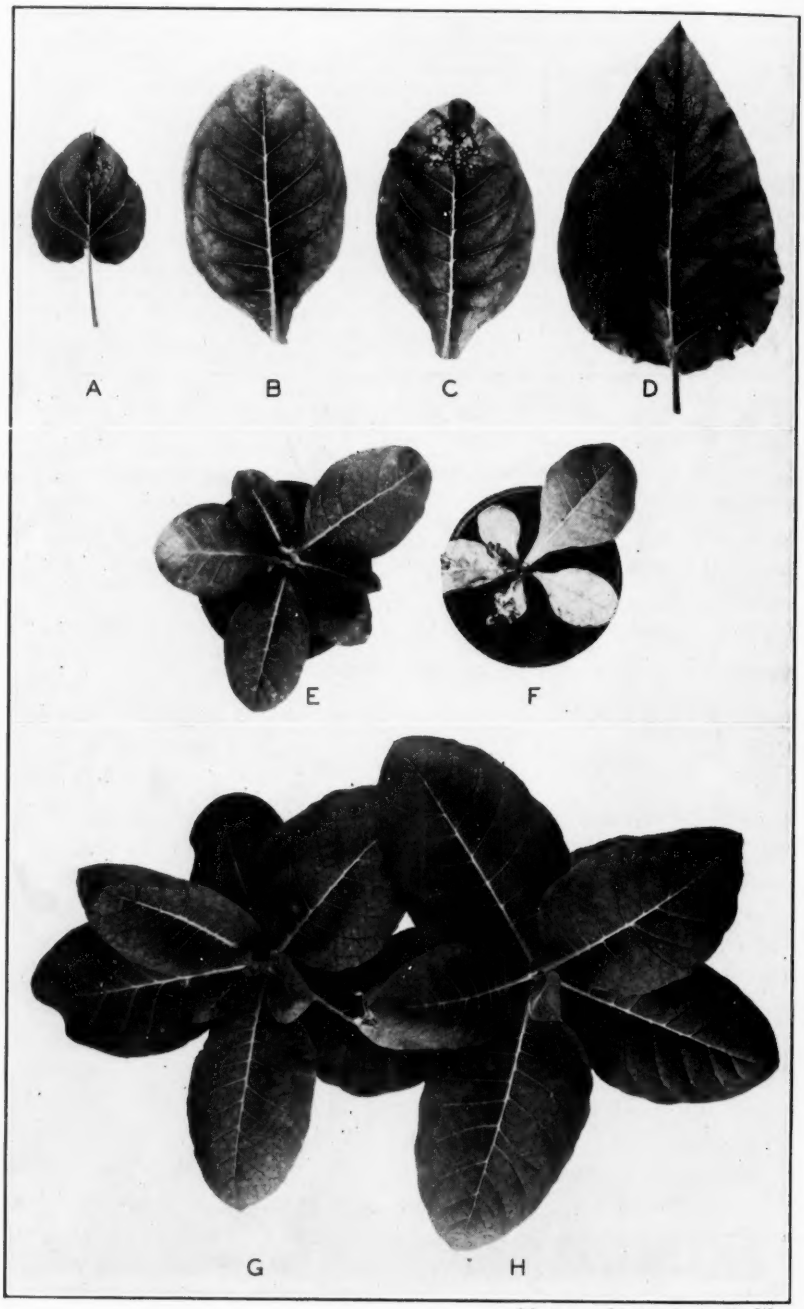
[b] Beginning with a necrotic-type plant of B_1 as origin in each series, a series of filial generations was produced, using a necrotic-type plant of the preceding generation as parent in each case.

[c] Because *N. digluta* itself originally had *N. tabacum* as one parent, and carried a full set of *N. tabacum* chromosomes, the numbers in this column are larger by one than is usually the case for hybrids of the indicated generations.

introduction from *Nicotiana glutinosa* of a single dominant gene inducing necrotic-type response to infection by tobacco-mosaic virus. This gene will be referred to as *N* (necrotic-type response to infection with tobacco-mosaic virus), and its recessive allele, characteristic of *N. tabacum* and of chlorotic-type derivatives of the *N. digluta-tabacum* cross, will be referred to as *n* (chlorotic-type response). Only 2 disease types were observed in the entire series of generations; these were the necrotic and chlorotic types, characteristic of *N. glutinosa* and *N. tabacum*, respectively. No difference was observed between the response of heterozygous (*Nn*) plants and that of homozygous (*NN*) plants. No modification of the action of the *N. glutinosa* gene *N* was detected at any time, despite the varied genetic constitutions of the hybrids into which it was introduced.

In the Burley backcross line, segregation of a pair of genes, presumably the genes G_1g_1 or G_2g_2 of Henika (5), controlling green *vs.* white leaf color, was found to be independent of that of the pair of genes *Nn* controlling necrotic *vs.* chlorotic type of disease. The observed ratios were 71 green necrotic : 69 white necrotic : 70 green chlorotic : 70 white chlorotic, among progeny from doubly heterozygous green necrotic ♀ and doubly recessive white chlorotic ♂ parents.

Throughout this investigation it was found that spread of virus occurred about as promptly in the very youngest necrotic-type plants as in comparable chlorotic-type plants. When early diagnosis of disease type was desired and plants were also to be saved for production of seed, the severity of the sys-



Photographs by J. A. Carlile.

FIG. 1. Leaves 5 days after inoculation with distorting-strain tobacco-mosaic virus, and plants 14 days after similar inoculation. A. From *NN* plant of *Nicotiana glutinosa*. B. From *nn* plant of *N. tabacum*, Burley backcross. C. From *Nn* plant of same. D. From *NN* plant of the repeatedly selfed Samsoun tobacco line; note necrotic lesions in all leaves of plants possessing the dominant gene *N*. E. Young *nn* Burley backcross plant showing *systemic chlorosis*. F. Similar young *Nn* plant showing *systemic necrosis;* contrast with E. G. Older *nn* plant showing systemic chlorosis. H. Comparable *Nn* plant showing only *local necrosis* at site of inoculation. E–G. Inoculated leaf at left.

temic necrosis proved troublesome. It was found, however, that, as plants became slightly older, there was an increasing delay of systemic infection in the presence of the gene for necrosis. It was then possible, after inoculating, to allow systemic chlorosis to appear in all *nn* plants before safeguarding necrotic-type plants by cutting away the inoculated leaves to prevent subsequent escape of virus into stems and top leaves. In still older plants inoculated leaves could be left attached indefinitely without escape of virus to other parts.

Observed deficiencies of necrotic-type plants in progenies derived from Connecticut Broadleaf tobacco, as shown in the third column of table 1, are not entirely understood. Successive generations have shown no indication of any tendency toward establishment of the gene *N*. No simple 1:1 backcross or 3:1 selfed ratios have occurred thus far. It seems probable, however, that a subsequent approach from a different angle, by hybridization of the necrotic-type Burley and Samsoun derivatives with Connecticut Broadleaf tobacco, may permit the necrotic-type gene to be incorporated in this third variety also.

In figure 1, leaves and plants of necrotic and chlorotic types are represented as they appeared at certain intervals after inoculation. The tendency to virus localization in necrotic-type plants is illustrated.

HOMOZYGOUS NECROTIC-TYPE LINES OF NICOTIANA TABACUM

After the first backcross generation there was little visible indication of any characteristic of *Nicotiana glutinosa* in the hybrids. In successive generations of crosses of necrotic-type plants with 3 varieties of *N. tabacum*, the distinctive characteristics of the 3 horticultural types were soon acquired.

For the purpose of obtaining plants homozygous with respect to the gene *N*, selfed lines were instituted with necrotic-type plants of the first backcross generation as origin. No homozygous sets of plants were attained in the Connecticut Broadleaf selfed line, as might be expected from the failure of this line to produce normal monohybrid ratios. The selfed line of Burley yielded satisfactory 3:1 ratios, but this also has not yet given any progenies lacking chlorotic-type individuals. In the selfed line of Samsoun tobacco, however, sets consisting only of necrotic-type plants were obtained. These obviously were derived from homozygous parent plants, in gametes of which the chromosome bearing the gene *N* had been included regularly enough to avoid formation of homozygous recessive-type plants in the tested sample of progeny. Whether complete gametic purity with respect to the gene *N* had been attained was an important question. A more sensitive test of this was given by reciprocal backcrosses to a chlorotic-type tobacco plant.

The apparently homozygous necrotic-type (*NN*) Samsoun tobacco derivative, which produced 339 necrotic-type : 0 chlorotic-type plants in the F_3 generation (Table 1), was crossed reciprocally with a plant of the original chlorotic-type variety Samsoun (*nn*), using an individual of the F_3 generation first as pollen parent, then as seed parent. Selfed progeny of the Sam-

soun plant were found to be consistently of chlorotic type (250 individuals tested). Selfed progeny of the F_3 plant were those reported in the table as the F_4 generation (303 individuals tested, all found to be of necrotic type). In each of the reciprocal crosses, 250 plants of the progeny were tested by inoculation. Without exception these proved to be necrotic-type plants. This constituted a critical test of 250 male and 250 female gametes, all of which were thus found to carry the newly introduced gene *N*. A single failure among the 500 would have been disclosed by the appearance of a chlorotic-type plant. To have made an equally sensitive test of gametic purity by self-pollination would have required the square of this number, *i.e.*, 250,000 plants, since the detection of 1 failure in 500 gametes would have been possible only if that gamete happened to fertilize, or to be fertilized by, a similarly rare gamete also failing to carry the gene *N*. The demonstration of gametic purity of the homozygous *NN* line of Samsoun tobacco is thus very satisfactory.

It is believed that similar homozygous lines corresponding to locally desirable horticultural varieties of *Nicotiana tabacum* can be produced readily, either by repeated backcrosses of the homozygous stock of Samsoun tobacco here described, with desired types as recurrent parents, or better by similar crosses of necrotic-type, *Nn* plants of the B_4 generation described in this paper (see table 1). These B_4 plants possess the advantage of having been crossed repeatedly to *N. tabacum* varieties, with opportunity for crossing-over to have transformed the chromosome that bears the newly introduced gene *N* into an essentially *N. tabacum* type of chromosome.

DISCUSSION

Type of Resistance Conferred by the Gene *N*

Immunity from infection by tobacco-mosaic virus is unknown among species of the genus *Nicotiana*. There are, however, important differences in type of response to infection within the confines of the genus (6). Some *Nicotiana* species tend to remain symptomless after they are infected; others show systemic chlorosis. Both the symptomless and the chlorotic-type species, once infected, retain the virus throughout their natural span of life, facilitating spread of virus by providing a good source of inoculum. Still other *Nicotiana* species show systemic necrosis or localized necrosis as a result of infection. The necrotic-type species are protected from plant-to-plant spread of all strains of the virus by early death of invaded tissues, with consequent imprisonment of most of the virus. Their response constitutes an effective type of resistance to the disease from a practical viewpoint, in the sense that spread of the disease through the population is greatly impeded. This particular kind of resistance occurs also among other solanaceous plants, as, for example, in some varieties of eggplant, *Solanum melongena* L. Thus, the Black Beauty eggplant dies as a result of systemic necrosis if infected when young, but localizes the virus in necrotic primary lesions if infected when older (6, p. 333). Under field conditions, injury to this

necrotic-type eggplant is unknown, apparently because no strain of the virus becomes established in large amounts within the crop. Valleau (12) has referred to this type of resistance as depending on *sensitivity* with respect to the virus.

In the past there have been no necrotic-type varieties of tobacco, *Nicotiana tabacum*. All varieties except the symptomless Ambalema tobacco and its derivatives (10, 11) have shown the classical mottling type of systemic chlorosis after infection with ordinary tobacco-mosaic virus. Very large amounts of virus develop in the infected chlorotic-type plants. Consequently, these plants serve during the remainder of their life, and later, when dried, as the principal reservoir from which comes the virus for contaminative infection of later crops of tobacco and other susceptible cultivated species. It is to combat this accumulation of virus in the tobacco crop, rather than to protect the individual infected plant, that a necrotic-type response may prove useful if it can be introduced into horticulturally acceptable strains of tobacco. In the pepper, *Capsicum frutescens* L., complete localization of tobacco-mosaic virus occurs at ordinary temperatures in all plants bearing a dominant gene *L* (7, 8). This localization is permanent because the inoculated leaf is lost by abscission soon after the appearance of necrotic lesions at the site of inoculation. The infected individual is adequately protected. This must not be expected in necrotic-type tobacco, for, although all strains of tobacco-mosaic virus elicit the necrotic type of response, leaf abscission does not follow infection. Both the degree of localization of virus that occurs in old plants and the death of young infected plants, however, would be efficacious in preventing the development of large amounts of inoculum in tobacco. The symptomless condition in the variety Ambalema tends to serve the same purpose with less risk to individual infected plants, but is controlled by a more complex genetic system (4), and is believed to be less uniform in its response to different strains of the virus (12, p. 207).

In the course of the present investigation, a dominant gene for necrotic-type response to infection with tobacco-mosaic virus was incorporated in an inbred line of *Nicotiana tabacum*. This made available a necrotic-type strain of tobacco which is fully fertile with the innumerable tobacco varieties now grown.

The protecting gene is inherited as a Mendelian dominant. Its identification by inoculation methods is readily accomplished. It is hoped, therefore, that further work on the incorporation of the gene into locally acceptable strains of tobacco and subsequent trials under field conditions may be left largely to those who are especially interested in the maintenance and improvement of varieties of tobacco.

Earlier Recognized Functions of the Gene *N*

The dominant gene *N* of *Nicotiana glutinosa*, with which the present investigation is concerned, has played an important rôle in the study of tobacco-mosaic virus, in part even before its identity was recognized. Al-

though its effect in preventing the mottling manifestations of classical tobacco-mosaic disease was the first to be noted, by Allard in 1914 (1, p. 14), this function was not the first to be exploited. Its first important contribution to the scientific investigation of tobacco-mosaic disease was in facilitating quantitative measurement of the causative virus by inducing prompt formation of conspicuous primary lesions, which in great number on a given leaf surface indicated high titer of virus, but, in less abundance, indicated lower titer. A similar gene of *N. rustica* L. and a mechanism of unknown genetic nature in *Phaseolus vulgaris* L. also have been utilized for the same purpose. The ease of performing quantitative measurements thus gained has allowed unusually extensive as well as intensive studies of this virus to be carried out in the decade since 1928, the year in which this method of measurement began to replace earlier, but less efficient and less productive, techniques of minimal inoculation of *N. tabacum* and *N. rustica* plants. The second contribution of this dominant gene was in allowing accurate separation of strains of tobacco-mosaic virus. A similar gene of *N. langsdorffii* Weinm. has been used also for this purpose, permitting many newly derived strains to be promptly separated when they arose from strains already in hand. Its third contribution, in conferring a desirable type of disease resistance, though envisaged first and long delayed in accomplishment, may prove no less important through its apparently decisive control of epiphytotic spread of all strains of tobacco-mosaic virus in *N. tabacum*, the present outstanding source of contaminative infection for susceptible hosts of this virus among crop plants.

SUMMARY

A dominant gene *N*, inducing a necrotic type of response to infection with tobacco-mosaic virus, was transferred from *Nicotiana glutinosa*, through the medium of the amphidiploid species *N. digluta*, into strains of the species *N. tabacum*. By repeated backcrosses of necrotic-type hybrids to *N. tabacum*, tobacco-like derivatives of necrotic type were produced. A homozygous line was then attained by repeated selfings. This homozygous line is self-fertile and fertile with other strains of tobacco. The introduced gene is regularly distributed to its gametes, and so to all individuals of its progeny, whether obtained by selfing or hybridization. It is anticipated that it may prove feasible to incorporate this gene in locally acceptable types of tobacco, to prevent spread of virus from plant to plant within the tobacco crop, and so to eliminate the reservoir of virus in tobacco and tobacco products, the usual sources of infection both for succeeding crops of tobacco and for other crops susceptible to tobacco-mosaic disease.

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EFFECT OF SODIUM CITRATE ON RELEASE OF CURLY-TOP VIRUS FROM ALCOHOLIC PRECIPITATE OF PLANT JUICE[1]

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INTRODUCTION

Certain phases of investigations on the chemical nature of resistance to curly top involved steam distillation of plant material in the presence of a small amount of sulphuric acid. *Atriplex semibaccata* Brown, *Chenopodium murale* L., and *Lycopersicon esculentum* Mill. were thus treated. The large volume of distillate that contained the plant acids was concentrated in each case under reduced pressure 1/20 the original weight of material used and these concentrated fractions were adjusted to pH 7.0.

Tests were made to determine whether or not these concentrated extracts would inactivate the curly-top virus. One ml. of juice from diseased beet plants was added to 4 ml. of each extract. These mixtures were allowed to stand at room temperature for a definite period, usually overnight. The amount of virus present in the mixture was then determined by the method worked out by Bennett.[2]

[1] Contribution from the United States Department of Agriculture, Bureau of Plant Industry, Division of Sugar Plant Investigations, Riverside, California.

[2] Juice was expressed from the severely diseased leaves of 5 to 15 beet plants and centrifuged. To 1-ml. aliquots was added an equal volume of 95 per cent ethyl alcohol. The resulting precipitate was thrown down by centrifugation and the supernatant liquid, discarded. The precipitate was washed once with 50 per cent alcohol, dried, and suspended in water and centrifuged. The supernatant liquid was made acceptable food for the leaf hoppers by adding sufficient sucrose to make a 5 per cent solution. The percentage of leaf hoppers that transmitted the virus from such a liquid to seedling sugar beets indicated

The concentrated fraction from *Atriplex semibaccata* completely inactivated the virus immediately after being prepared. After 11 days of storage in a frozen condition, the same extract[3] lost part of its power to inactivate the virus. When tested a third time, 21 days after the first test, it not only had lost its power to inactivate the virus but liberated large quantities of virus. Inoculation tests resulted in 5-fold increase in infections over the controls. Another extract from *A. semibaccata,* prepared in the same manner as described, apparently had no effect on the virus immediately after being prepared, but on standing 16 days caused an 8-fold increase in infections over that of the controls.

The extract prepared from the juice of the tomato plant liberated the virus from diseased-beet juice, causing a 2-fold increase over the controls. When the fraction from *Chenopodium murale* was tested, sufficient virus was released to double the amount of infection over that obtained in the control tests. Bennett (1) found that 4 parts of juice expressed from *Atriplex semibaccata* inactivated the virus in 1 day when mixed with 1 part of juice from diseased beets.

These results suggested that the curly-top virus is adsorbed by proteins or possibly other substances precipitated from beet-leaf juice by alcohol, and that the salts of the plant acids extracted by steam distillation may, under certain conditions, release large quantities of the virus held in the colloidal complex.

As water is all but nondissociated, extraction with water presumably would not change the electrokinetic properties of the colloidal complex containing the virus; but, if an electrolyte were used, the electrokinetic potential would be shifted and it might be so adjusted as to effect dispersal of more virus.

EXPERIMENTAL PROCEDURE AND RESULTS

Effect of Low Concentrations of Certain Electrolytes on the Amount of Virus Released from the Protein Complex

Sodium citrate, because of its trivalent anion, which is largely responsible for its marked influence on the electrokinetic potential, was selected as one of the electrolytes with which to extract the virus from the protein complex. Five-tenths per cent solutions of sodium citrate,[4] sodium acetate, and sodium bicarbonate were prepared for comparative study.

Alcoholic precipitates were prepared in each experiment from mixed juice of 10 to 12 severely diseased plants. This avoided the variation in available virus encountered when single plants are used.

Each of the prepared solutions was tested for its ability to liberate the virus from the alcoholic precipitate. After extraction and centrifuging,

the relative amount of virus in the liquid. Controls in each test were similarly treated, except that 4 ml. of water was added to the juice instead of the extract.

[3] A precipitate slowly formed in the extracts during storage in the refrigerator. When samples were removed for the subsequent tests, only the supernatant liquid was used.

[4] When corrected for the slight moisture content of the anhydrous salt, the concentration of sodium citrate in the solution was 0.438 per cent.

sufficient sucrose was added to the supernatant liquid to bring the concentration to 5 per cent. The liquid was then fed to nonviruliferous leaf hoppers and the customary procedure for inoculation followed. For control tests, alcoholic precipitates were extracted with water and treated in the same manner. A different source of virus was used for each of the 3 experiments shown in table 1.

TABLE 1.—*The effect of certain electrolytes on the amount of curly-top virus released from the alcoholic precipitates of juice from diseased sugar-beet leaves*

Experiment number	Electrolyte added to 5 per cent sucrose	Plants inoculated	Plants infected
		Number	*Number*
38	Control	20	10
	Sodium bicarbonate	20	0
	Sodium acetate	20	11
	Sodium citrate	20	18
	Equal mixture of all 3 salts	20	1
40	Control	20	3
	Sodium citrate	20	12
43	Control	20	0
	Sodium citrate	20	3

The results show that certain electrolytes may play an important part in the release of the virus from the alcoholic precipitate. Sodium citrate released sufficient virus to give double the percentage of infection obtained in the controls. Sodium acetate had no effect on the amount of virus released, while sodium bicarbonate had a depressing effect.

To determine what concentration of sodium citrate would liberate the maximum amount of virus, one liter of a 1.6 per cent solution (54.4 milli-

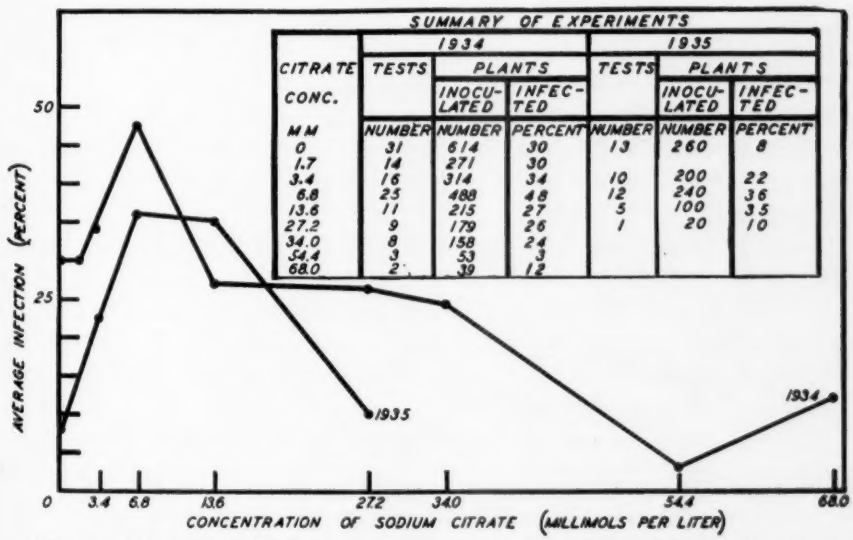

FIG. 1. Release of curly-top virus from alcoholic precipitate of beet-leaf juice with various concentrations of sodium citrate.

molar when corrected for the slight moisture content) of sodium citrate was prepared. Lower concentrations were prepared by diluting in turn 500 ml. of the next higher concentration with an equal volume of distilled water. The results of tests made over a period of 2 years with a range of concentrations of sodium citrate are presented in figure 1.

The percentage of infection reached an abrupt maximum. In the 1934 experiments, 6.8 mM sodium citrate was responsible for a 62 per cent increase in infection over the control tests.

Maximum percentage of infection was not at 6.8 millimolar concentration in all experiments. In 7 experiments in which 3.4 and 6.8 mM concentrations were used on the same source of virus, maximum infection was obtained with the lower concentration. In these 7 tests, the average percentage infection obtained with 3.4 mM was only 10 per cent more than the average values obtained with 6.8 mM sodium citrate.

These data were subjected to Student's method for interpreting paired experiments as modified by Love (6). Odds that the infection percentage obtained with 6.8 mM sodium citrate is significantly greater than that obtained by water extraction are greater than 9999 to 1 in the 1934 experiments. Odds that the 3.4, 6.8, and 13.6 mM sodium citrate concentrations were significantly greater with respect to infections obtained than was the case with water extraction were 132, 3332, and 124 to 1, respectively, for the 1935 tests.

On comparing the infection percentage of the tests conducted in 1935 with those of the previous year, it is evident that some factor, yet unknown, entered that greatly reduced the amount of infection. This is especially true in the control tests in which water was the extracting solution. The infection percentage in the control tests in 1935 was only one-fourth that of the control tests conducted in 1934. This difference cannot be attributed to the leaf hoppers, for all used were reared in the insectary by the regular method for producing nonviruliferous leaf hoppers (9).

Although only 5 experiments were conducted using 13.6 mM, it appears that in these tests (1935) in which the virus content was extremely low the concentration of sodium citrate releasing the greater amount of virus may lie between 6.8 and 13.6 mM.

Despite the extremely low percentage of infection in the control tests of 1935, the infections, as shown by the average percentages obtained for the 3 lower concentrations of sodium citrate in these tests, are even more striking than those obtained in 1934. In the 1935 tests, increases in the percentage of infection of 180, 475, and 462 per cent for the 3.4, 6.8, and 13.6 mM concentrations, respectively, were obtained.

Sodium citrate in concentrations greater than 13.6 mM reduced the amount of infection below that of the control tests. This cannot be ascribed to its effect on the leaf hopper because leaf hoppers were unaffected by feeding upon sodium citrate concentrations as high as 54.4 mM. Mortality of the leaf hoppers was low during the feeding period and equally distributed over the controls and all the sodium citrate concentrations used.

In order to consider the virus-releasing ability of sodium citrate as a function of the amount of virus released by water, the foregoing experiments were placed in 4 groups, according to the percentages of infection obtained with water extractions. The first group (A) consisted of all experiments in which the infection percentage in the control tests was 10 or below. Group (B) contained those experiments in which the control tests ranged from 11 to 20 per cent infection. Group (C) contained those experiments in which the control tests ranged from 21 to 40 per cent. All experiments in which the infection percentage in the control tests was above 41 constituted the fourth group (D).

Within each group, the ratios of the average percentage of infection obtained by the various citrate concentrations to the average percentage of infection of the control tests are plotted against the citrate concentrations. The results of thus grouping the experiments are shown in figure 2.

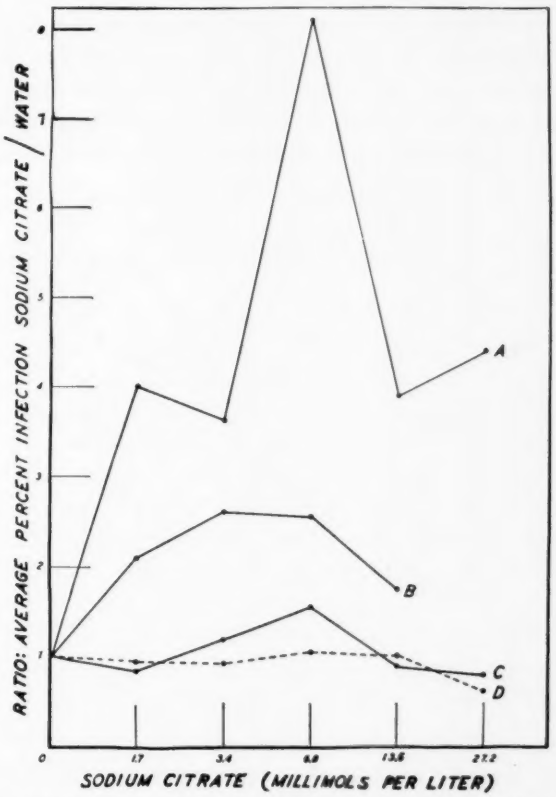

FIG. 2. Data from figure 1, showing ratios of infection percentages, sodium citrate to water, grouped as follows: A, infection in controls less than 10 per cent; B, infection in controls 11 to 20 per cent; C, infection in controls 21 to 40 per cent; and D, infection in controls 40 per cent or above.

Calculations (6) show that the infection percentages using 6.8 millimolar concentrations of sodium citrate were significantly greater than those for

water extractions in groups A, B, and C. The odds favoring 6.8 millimolar sodium citrate are greater than 9,999 to 1 for groups A and B, and 66 to 1 for group C. For group D, the two methods of extraction did not differ.

The increase in percentage of infection with sodium citrate extracts relative to water extracts becomes much greater as the amount released by water decreases. Where the virus is apparently so strongly adsorbed by the precipitate that water is only capable of liberating sufficient virus to give 10 per cent infection, sodium citrate is very effective in liberating the virus. In these tests, 6.8 mM sodium citrate liberated sufficient virus to increase the average percentage of infection 8-fold over that obtained with water. Of the 15 control tests of group A, 7 failed to give infection. In 4 experiments in which no infection was obtained in the control tests, 6.8 mM sodium citrate gave 50, 50, 30, and 20 per cent infection.

Group B, in which 10 and 20 per cent infection was secured from the water extract, the virus was still apparently adsorbed by the precipitate, since sodium citrate increased the percentage of infection 2.5-fold. It appears, from the experiments in which the control tests gave 21 and 40 per cent infection (group C), that the virus was adsorbed in those cases only to a limited extent. In this group, 6.8 mM sodium citrate increased the infection percentage only 1.5-fold above that of the controls. In those experiments in

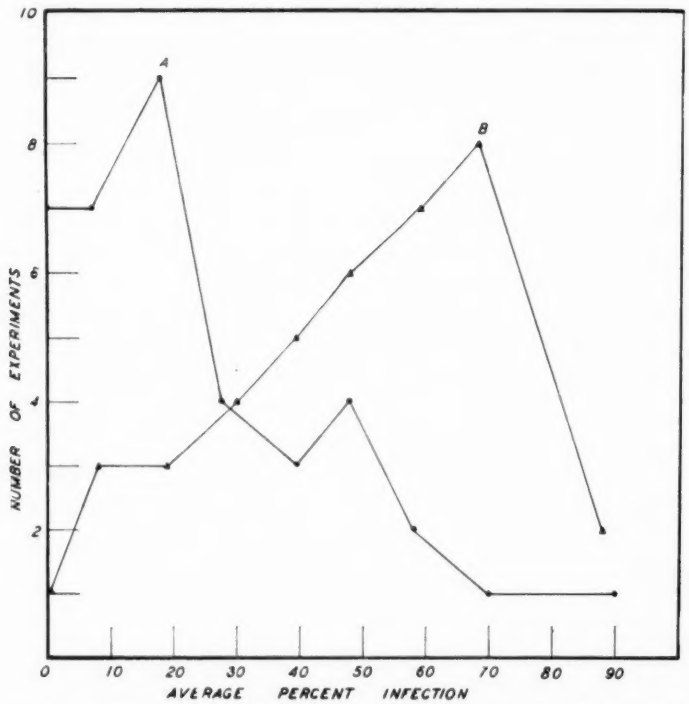

FIG. 3. Distribution of the experiments with respect to amount of infection obtained: A, experiments in which virus was extracted from the alcoholic precipitate with water; B, experiments in which sodium citrate was used to extract virus.

which water was capable of liberating sufficient virus to cause greater than 41 per cent infection (group D), no increase in infection was noted where sodium citrate was used as the extracting solution.

To point out further the effectiveness of sodium citrate in comparison with water as a releasing agent, the data of the experiments were separated as to extraction method and grouped according to the percentages of infection obtained. The groups were as follows: 0, 1 to 10, 11 to 20, 21 to 30, 31 to 40, 41 to 50, 51 to 60, 61 to 70, and 71 to 90 per cent. The average percentage infection of each group was then plotted against the number of experiments falling within each group (Fig. 3). Where water was used as the extracting solution, 55 per cent of the experiments fell in the first 3 groups, whereas only 18 per cent of the experiments in which sodium citrate was used as the extracting solution fell in these same groups and 59 per cent of the experiments fell in the 4 highest groups. There were 7 experiments out of a total of 38 conducted over a period of 2 years (a different source of virus for each experiment) in which no infection was obtained when water was used for extraction. Such results, if only water extractions had been used, would be taken to indicate that no virus was present in approximately 18 per cent of the experiments, but in these experiments the virus was demonstrated to be present with sodium citrate as the extracting agent. While water failed to give infection, infection with sodium citrate varied from 5 to 70 per cent, with an average of 26 per cent.

Effect of Hydrogen-ions on the Release of Virus by Sodium Citrate

Bennett (1) subjected the virus to various hydrogen-ion concentrations ranging from pH 2.4 to 9.1 by adding 1 volume of phloem exudate, containing the virus, to 4 volumes of buffered solutions. After the mixture stood 2 hours, a sample was removed for pH determinations and the remainder was fed to nonviruliferous beet-leaf hoppers 4 hours. Thus, the virus was subjected to the hydrogen-ion activity of the medium for a minimum period of 2 hours and a maximum period of 6 hours. He obtained no infection when the virus was subjected to a pH less than 3.4. Only 5 per cent infection was obtained at pH 3.4. In another experiment, pH values below 4.0 apparently inactivated the virus.

Bennett (1) also found that 4 volumes of juice of *Oxalis corniculata* L. inactivated the virus when mixed with 1 volume of juice from diseased beets (pH of mixture, 2.3–2.5) in about 30 minutes. The virus remained in contact with the juice for a definite period and then was precipitated with alcohol and removed from the presence of the acid by centrifuging.

A short exposure to a comparatively low hydrogen-ion concentration might not destroy the virus, but merely cause it to be adsorbed or fixed by the plant proteins in such a way that little or no virus would be liberated by extraction with water. Experiments, therefore, were conducted to compare the ability of sodium citrate and water to liberate virus that had been subjected previously to various hydrogen-ion concentrations. Using 1-ml.

aliquots of juice extracted from diseased beet leaves, various amounts of sulphuric acid were added to bring the pH to definite values. Each sample of treated juice was shaken and allowed to stand for 15 minutes. It was then precipitated and treated in the usual manner.

The beef-leaf juice was adjusted to a pH value of 4.7 in 5 experiments and to 4.1 in 7 others. Inasmuch as the amount of infection was in the same general range for both pH values, the data obtained are combined. Tests were made in 1934 and repeated in 1935. The results for the two years are shown in figure 4.

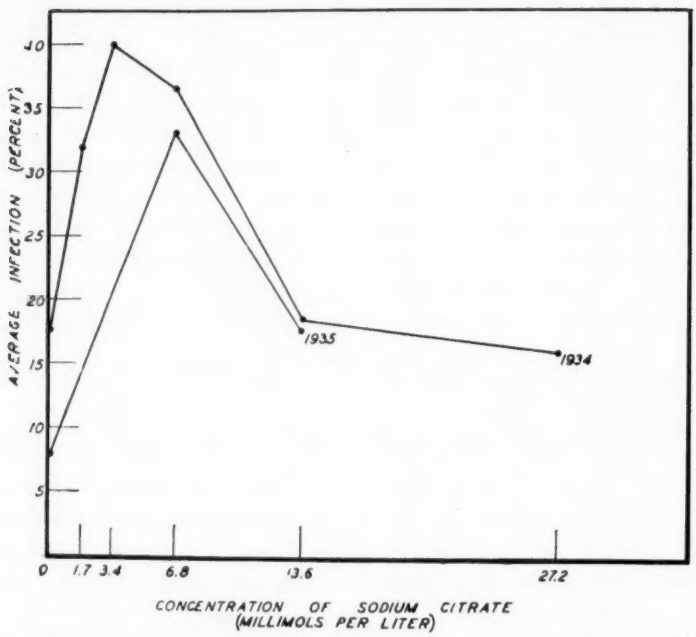

FIG. 4. Release of curly-top virus by various concentrations of sodium citrate from the alcoholic precipitate of beet-leaf juice adjusted to pH values of 4.1 and 4.7.

Here, as in figure 1, sodium citrate liberated more virus than did water. In these tests the 3 lowest concentrations appeared to be approximately equal in their ability to liberate the virus. It is evident that the exposure to pH 4.1 or 4.7 caused a reducion in the amount of virus extracted by water and that sodium citrate counteracted this effect, since the infections obtained reached levels comparable with those of former experiments.

Tests also were made to determine the liberation of virus from samples of juice from diseased beet leaves that had been subjected to higher hydrogen-ion concentrations. The data are presented in table 2. In Experiment 4, juice of Oxalis was used to adjust the pH. It is evident that exposures of the virus (in beet-leaf juice) to hydrogen-ion concentrations of pH 3.7, 3.4, and 2.2 the amount of virus extracted by water was decidedly below that shown for the nontreated juice (pH 6.2). When sodium citrate was used to extract the virus, the percentages of infection rose sharply, exceeding in

TABLE 2.—*Effect of certain hydrogen-ion concentrations on the virus in the expressed juice from beet leaves affected with curly top*

Experiment number	Reaction to which the plant juice was adjusted before precipitation	Infection secured from alcoholic precipitate following extraction with:			
		Water	Sodium citrate at indicated concentrations		
			3.4 mM	6.8 mM	34 mM
	pH	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	6.2 (normal)	50	70	50	.....
	3.7	0	60	55	.....
	3.4	15	80	20	.....
2	6.2	0	10	.....	.....
	3.7	0	20	.....	.....
	3.4	0	10	.....	.....
3	6.2	30	.....	.....	25
	2.2	0	.....	.....	30
4[a]	6.2	55	.....	70	.....
	2.5[b]	.....	.....	20	.....
	2.5[c]	.....	.....	60	.....

[a] Allowed to stand for 16 hours in the presence of the acid.
[b] Brought to pH 2.5 by the addition of Oxalis juice.
[c] An equal volume of 95 per cent alcohol and sufficient Oxalis juice was added to reduce the pH to 2.5.

many cases the percentages obtained when the juice was not exposed to high hydrogen-ion concentrations. It is believed that the virus is not destroyed at the lower pH values but only fixed or adsorbed.

In another test the virus was subjected to 0.1 normal sulphuric acid for a period of 2 hours. Following this exposure, the virus was precipitated from 3, one-ml. aliquots of juice and washed in the usual manner. One of the precipitates was extracted with 6.8 mM sodium citrate. Fifteen per cent infection was obtained with this extract. The two remaining precipitates were resuspended in 2 ml. of 0.1 normal sulphuric acid and allowed to stand, with frequent shaking, for 2 hours. The acid was neutralized with 2 ml. of 0.1 normal sodium hydroxide and the virus was again precipitated with alcohol, washed, and dried in the usual manner. One of the precipitates was extracted with water, while the other was extracted with 6.8 mM sodium citrate. These extracts were tested for the presence of virus, with the result that no infection was obtained with water, whereas 10 per cent infection was obtained with sodium citrate. From this experiment, it appears that the virus is not destroyed by brief exposures to hydrogen-ion concentrations equivalent to approximately pH 1.

Release of Virus from Tomato-Plant Juice Adjusted to Various Hydrogen-ion Concentrations

Tests were made to determine if sodium citrate is capable of liberating the virus from the alcoholic precipitate of the juice of other curly-top-susceptible plants. Diseased tomato plants were frozen and the juice was

extracted and centrifuged. The virus was precipitated from 1-ml. aliquots of juice with alcohol, washed, and dried in the usual manner. The virus then was extracted with water (for the control test) and with the usual concentrations of sodium citrate. Tests also were made to determine if sodium citrate is capable of liberating the virus from tomato-plant juice that had been previously adjusted to a pH value of 4.1. The methods used in these experiments were the same as those previously described. The results of the experiments in which diseased tomato plants were used as the source of virus are summarized in figure 5.

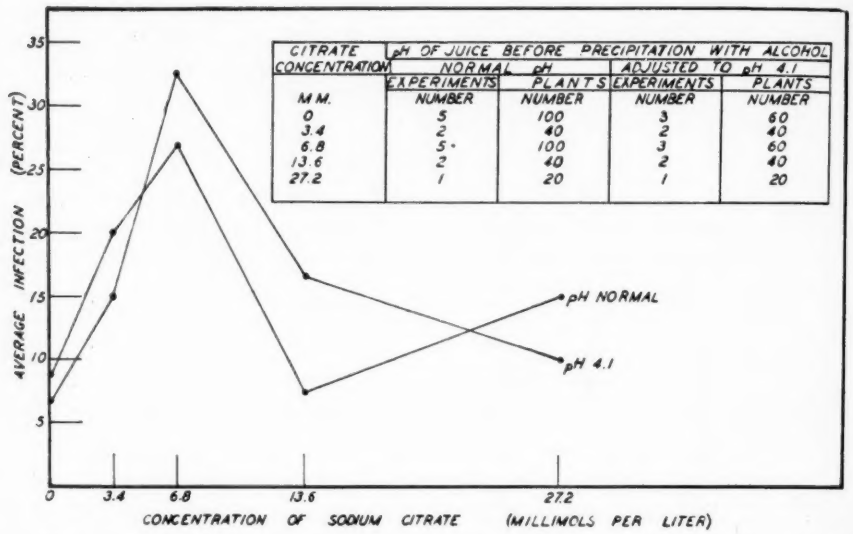

FIG. 5. The release of curly-top virus from alcoholic precipitate of tomato-plant juice with water and with various concentrations of sodium citrate.

It appears that the same factors influencing the binding of the virus in the precipitate from beet-leaf juice are operative in the precipitates from juice of diseased tomato plants. The maximum amount of infection was obtained with 6.8 mM of sodium citrate when the tomato plant served as the source of virus.

The experiments illustrated in figure 5 were conducted in 1935 concurrently with those represented by the lower curve of figure 1. For some reason, in 1935, little virus was released from the alcoholic precipitate when water was used for extraction, regardless of the source of virus (Figs. 1 and 5). In the 1935 experiments sodium citrate (6.8 mM) was, however, capable of liberating sufficient virus from the alcoholic precipitate of either beet-leaf juice or tomato-plant juice to cause a 4-fold increase over that obtained with water.

Subjecting the virus in the tomato-plant juice to a hydrogen-ion concentration of pH 4.1 apparently did not greatly influence the amount subsequently released by either water or sodium citrate.

The Peptization of the Proteins in the Alcoholic Precipitate of Beet-Leaf Juice by Different Concentrations of Sodium Citrate

Gortner, Hoffman, and Sinclair (5) demonstrated that the protein complex of wheat flour could be peptized to different degrees when treated with different inorganic salt solutions. Sinclair and Gortner (8) studied the physico-chemical behavior of a pure protein (gliadin) when treated with neutral salt solutions. These investigators found that the amount of protein (gliadin) that will be peptized by a particular salt solution depends both upon the concentration of the salt solution and the amount of gliadin present.

Experiments were conducted to determine if any correlation exists between the amount of protein brought into solution by extracting the alcoholic precipitate from beet-leaf juice with the various concentrations of sodium citrate and the amount of virus released by the same concentrations.

Leaves from diseased beet plants were collected and frozen. The juice was expressed and the coarser particles allowed to settle. Two hundred ml. aliquots of juice were pipetted off the top, adjusted to pH 4.7, and sufficient alcohol was added to bring the concentration to 50 per cent. The mixture was allowed to stand overnight at room temperature, and then the precipitate was filtered off and washed once with 50 per cent alcohol.

The precipitates were ground in a mortar in the presence of small amounts of the extracting solution. Grinding was continued until the volume in each case was brought to 200 ml. Extraction was allowed to proceed at room temperature for 24 hours, then the extracts were filtered through paper pulp until a clear liquid was obtained. The amount of nitrogen in the filtrate was taken as a measure of the amount of protein peptized by the various concentrations of sodium citrate used. The data are shown in figure 6, together with the amount of infection obtained at the same concentrations.

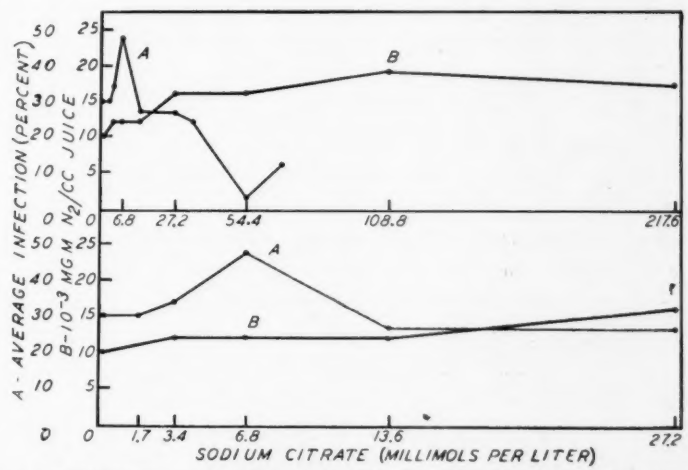

FIG. 6. Peptization of proteins (curve B) and amount of infection (curve A) obtained with the same concentrations of sodium citrate. Results at lower concentrations also plotted on larger scale.

It is noted that the maximum amount of infection was obtained at 6.8 mM sodium citrate, whereas the maximum amount of proteins were peptized by a concentration of 108.8 mM sodium citrate (Fig. 6). Obviously, the amount of protein peptized by the various concentrations of sodium citrate bears no direct relation to the amount of infection obtained.

DISCUSSION

The method developed by Bennett (1) for testing the presence of virus and, from this, obtaining an estimate of the amount present, although greatly improved over the method of Carter (3) and Severin and Swezy (7), is not completely reliable. Extraction of the alcoholic precipitates with water gives variable results, especially where the amount of recoverable virus is low. Extraction with sodium citrate gives more dependable results.

Inasmuch as all proteins exhibit electrokinetic phenomena (2), (4), it was postulated that the virus may be adsorbed under certain conditions on proteins or other substances in the colloidal state that are precipitated from beet-leaf juice along with the virus upon the addition of alcohol. If this were true, the alcoholic precipitate might contain large quantities of virus so completely adsorbed by the proteins that little or none of it would be liberated from the protein-virus complex by extraction with water. Experiments were undertaken in an attempt to develop a method that would release the virus and thereby increase the amount of infection.

The results of the experiments reported strongly suggest that some protein or other substance present in the juice of sugar beets, tomatoes, and probably other plants adsorbs the virus with such avidity that water is incapable of liberating it. It seems probable that when this adsorbing complex is saturated with virus and an excess of virus left free, then extraction of the alcoholic precipitate with water may give an indication of the amount of nonadsorbed virus present rather than the total amount present. Those experiments in which no infection was obtained when water was used for extraction may be accounted for by complete adsorption of the virus. When the same precipitates were extracted with sodium citrate, infection was obtained, which indicates definitely a release of the curly-top virus.

In attempting to explain the mechanism by which the virus is released from the plant protein, or other substances, probably colloidal in nature, the theory of Helmholtz, involving the presence of an electric double layer in the immediate vicinity of the colloidal micella, must be taken into consideration. It is known, for example, that different proteins under the same conditions may exhibit different properties, depending upon the magnitude and sign of the charge on the micellae. It is possible that the micellae of 2 proteins in the colloidal state under the same conditions may possess opposite charges. If the micellae of plant proteins possess a strong charge, they would attract and hold ions and micellae of the opposite charge. The principal force by which the curly-top virus is closely held by the plant proteins, or other substances in the precipitate, may be allied to these electrokinetic phenomena.

The effect of certain ions on the electrophoretic mobilities and the sign of the charge carried by proteins is well demonstrated by Tiselius (10). The addition of a non-electrolyte, such as water or sucrose, would neither reduce nor increase the charge set up at the electric double layer; consequently, it would have little effect upon the adhesive forces binding the virus to the plant proteins or upon the forces holding the particles together. The addition of an electrolyte would, however, affect profoundly the properties exhibited by the different micellae in the system.

SUMMARY

Preliminary experiments indicated that curly-top virus may be so intimately tied up with the plant proteins and other substances precipitable by alcohol that water is unable to release sufficient quantities of virus to give consistent amount of infection. Sodium citrate, however, which ranks at the top of the lyotrophic series, proved very effective in releasing the virus.

Maximum infection was obtained when the alcoholic precipitate was extracted with 6.8 mM sodium citrate. Concentrations greater than this amount generally reduced the percentage of infection.

It appears that where the extractable virus content of the plant juice is low, as shown by the percentage infection obtained in the control tests, the sodium citrate method of extracting the virus is far superior to the method in which water alone is used, and a part of the variability in results avoided.

When the pH of the extracted beet leaf juice containing the virus was reduced below 4.1, prior to precipitation with alcohol, limited infection was obtained if water was used for extraction. The virus, however, is not irreversibly inactivated at these pH values or at values as low as pH 2.2, because infection was obtained by extracting the alcoholic precipitates obtained after such exposures with sodium citrate. The virus apparently was only fixed in such a way that water is incapable of liberating it from the precipitate.

It appears that the same factors that fix or adsorb the virus in the juice from diseased beet leaves may also be operative with the virus in juice from the tomato plant, since the maximum percentage of infection with both plant juices was obtained after extraction of the precipitates with 6.8 millimols of sodium citrate.

Subjecting the virus in the tomato plant juice to a hydrogen-ion concentration of pH 4.1 had no definite effect on the amount subsequently released by water or sodium citrate.

The hypothesis is offered that these results may be explained on the basis of electrokinetic effects produced by the sodium citrate.

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THE COMPARATIVE IMPORTANCE OF LEAVES AND TWIGS AS OVERWINTERING INFECTION SOURCES OF THE PEAR LEAF-BLIGHT PATHOGEN, FABRAEA MACULATA

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INTRODUCTION

Leaf blight of pear and quince is a common disease in the eastern half of the United States, and at one time or another since 1854 (5) or earlier it has been reported from many of our States (6, 8, 12, 13, 14, 15, 17, 18, 19, 22, 24, 25, 29, 30, 35, 36, 38, 39, 40, 41, 47, 48, 49, 50). It is often a serious disease of pears and quinces grown in poorly sprayed orchards and in home gardens. It has also been reported from Canada (1, 21, 27, 31), and is well known in New Zealand (7, 20), Australia (9, 26), South America (16), South Africa (10, 11), Asia (28, 45, 51), and in Europe (23, 32, 33, 37, 42, 43, 46).

It has also been described on *Sorbus* sp. (2, 8), *Amelanchier* sp. (49), *Aronia* sp. (37), *Cotoneaster* sp. (33), and *Photinia* sp. (46).

OVERWINTERING OF THE ORGANISM ON DISEASED LEAVES

Since Atkinson (3, 4) collected and described the type material of *Fabraea maculata* (Lév.), the importance of the ascigerous stage in the dissemination of the disease from year to year has been emphasized (34, 42, 43). Although pathologists (7, 12, 39, 44) know that the ascigerous stage is not very common, none has made any attempt to find out whether or not this stage is essential to the overwinering of the fungus. For several years the writers (39) have made a diligent search for perithecia and acervuli on overwintered leaves in Maryland and Missouri, but have found no perithecia and only a few conidia. To determine whether or not fallen diseased leaves were of importance in carrying the pathogen over from season to season, several sets of experiments were carried out in Maryland and Missouri.

In one set of experiments diseased leaves were taken from beneath diseased trees and placed under trees that had been kept free of the disease by spraying. In collecting diseased leaves from beneath certain trees it was possible, because of the absence of diseased neighboring trees, to remove all the diseased leaves from under and around the experimental trees. In this experiment, therefore, trees not previously diseased were exposed, and those previously diseased were not exposed to diseased leaves. If the disease developed in the first case, then diseased leaves were responsible. If it developed in the second, diseased leaves were not responsible. The trees used in this experiment were not sprayed during the following growing season. Experiments of this type were conducted during the 1935 and 1936 seasons in Maryland and Missouri. Tables 1 and 2, respectively, show the results obtained at Beltsville, Md., and at the Missouri stations in 1935 and 1936. Fruit infection was negligible on the Beltsville trees, exposed to diseased leaves for the first time, but was plentiful both on trees that were deprived of diseased leaves and on trees under which the diseased leaves were allowed to remain. At the Missouri stations primary leaf-blight did not occur on trees deprived of their bark cankers and diseased leaves, and infections were delayed on trees deprived of the bark cankers but exposed to diseased leaves. At Beltsville one tree previously free from the disease was exposed to diseased leaves for 2 years, but only a very few leaves became infected.

TABLE 1.—*Relation of diseased leaves beneath disease-free pear trees to incidence of leaf blight in such trees and also the effect of removal of blighted leaves from beneath diseased trees on the recurrence of leaf blight in those trees*

No. of trees	Treatment	Year	Fruits counted	Diseased	Diseased
			Number	*Number*	*Per cent*
2	Diseased leaves placed under	1935	794	7	0.9
4	do	1936	2974	28	0.9
1[a]	do	1935	1287	5	0.4
		1936			
1	Diseased leaves taken from	1935	793	33	4.16
1	do	1936	2252	695	30.9
1[b]	do	1935	725	461	63.6
		1936			
4	Diseased leaves left under	1935	5939	2021	35.6
4	do	1936	9781	2687	37.5

[a] Diseased material placed beneath the same tree during two seasons.
[b] Diseased material removed from beneath the same tree during two seasons.

During these 2 years of studies on the effect of overwintered leaves on the carryover of the leaf blight, other observations were made that confirm the results of these experiments. Several thousand pear seedlings, severely affected during their previous growth history by the leaf-blight organism, were moved in the winter of 1934 from the Arlington Experiment Station grounds in Virginia to a site at the United States Horticultural Station at Beltsville, Md., where no pear trees previously had been grown. Very little leaf material accompanied their transfer. The following spring, soon after

the new leaves appeared on these seedlings, typical leaf blight developed throughout the planting and caused severe early defoliation. Bark cankers developed plentifully during the season, and in the spring of 1936 outbreaks of primary leaf blight were traced back to these cankers. In all cases examined one or more cankers could be found just above the group of infected leaves.

Following the mild winter of 1936–37 at Beltsville, Md., many diseased leaves were found harboring viable conidia during the growing season of 1937. A number of these were placed in several wire baskets among presumably susceptible pear and quince leaves during rainy periods, and in each case a few typical leaf-blight lesions were produced on leaves just below the diseased leaves. In no case were lesions formed on leaves above the sources of inoculum. From this evidence, it appears that the few infections that developed were caused by conidia and not by ascospores, because if ascospores had been the responsible agent the infections would have occurred more generally and on leaves above as well as below the source of inoculum. It is possible, of course, that the lesions may have developed from chance infections due to conidia and ascospores in the orchard environment. It is unlikely, however, that this was true, since the observed lesions always were located just below the diseased leaves. Furthermore, despite extensive search, no asci have been found in diseased leaves collected in this orchard. On the other hand, viable conidia were known to be present on these diseased leaves. Since the leaf-phase experiments appear to demonstrate that diseased leaves are not commonly of much importance as overwintering infection sources, studies on the fruit and bark phases of the disease were initiated.

OVERWINTERING OF THE ORGANISM ON DISEASED FRUITS

The fruits of pear are severely affected by the leaf-blight organism and many of the diseased fruits are discarded and allowed to remain on the ground. Since lesions on these fallen diseased fruits persist for a long time, it was thought that possibly the pathogen might overwinter in them. Conidia have been found on diseased fruits during the winter months, but no signs of an ascigerous stage have been observed. During the fall of 1935 and 1936 diseased fruits were collected and placed under trees that had previously been free from the disease. None of these trees became diseased during the two seasons. The diseased fruits slowly disintegrated during the winter and at the time the disease usually becomes prevalent among the newly formed leaves in the spring, the old lesions in the fruit were hardly recognizable. No viable conidia as determined by germination tests, were ever observed on overwintered fruit at that season.

OVERWINTERING OF THE ORGANISM IN BARK CANKERS

Sorauer (42), who was probably the first to describe the bark cankers, was of the opinion that they furnished a possible overwintering source of conidia. Although others (6, 11, 12, 14, 18, 23, 26, 44) have pointed out the

possibilities of this source of inoculum, none have demonstrated the real importance of bark cankers and how conidia are produced in them.

During the preliminary stages of these investigations, which were primarily concerned with control, it was observed that the location of the first infected leaves differed from that which would be expected if the source of spores was confined to overwintered diseased leaves. The primarily infected leaves on check (unsprayed) trees were always localized in inverted-cone-shape areas, directly below bark cankers that were yielding viable conidia. Never was the infection general except at a time of the season when conidia were being disseminated from the primary lesions. The disease usually became visible on the young leaves by the middle of May, but viable conidia were found in acervuli on bark cankers of the previous season before any leaf infections were visible. Only a relatively small percentage of the observed bark cankers appear to become active during the following spring, but occasionally a few conidia are obtained from cankers that are 2 years of age.

After the new leaves become infected and conidia are produced, bark cankers resulting from these conidia may be formed on current-year growth at any time during the remainder of the growing season, provided the environmental conditions are suitable and the bark tissues are in an immature condition. Bark cankers may be formed in the early spring or even late in the summer, and both early- and late-formed bark cankers may be active the following spring. Most of the cankers, however, appear to be active for only a relatively short period, and the great majority of them not only do not produce conidia but remain very small or are healed out. Many small cankers occurring on twigs and watersprouts do not develop to the stage where they produce acervuli. At times, however, the infections are more deep-seated, and the fungus may be readily isolated from cankers of this type. Several cankers may coalesce to form lesions of several centimeters in extent from which the pathogen may be isolated during a period of several months. These cankers are usually black and are never of a regular size or outline; usually the conidia escape through cracks in the epidermis above the surface of the acervuli, probably caused by the increase in girth of the stems.

During 1935, 1936, and 1937 a special study was made of the canker phase of the disease, since studies of the leaf phase had indicated that this was not the important means of overwintering of the pathogen.

In the fall of 1935, at Springfield, Mo., many cankers were tagged for observation, some of which were formed early and some late in the 1935 growing season on both Kieffer and Garber trees. Collections were made during the fall and winter of 1935 and the spring of 1936. The collected material was fixed in formalin-acetic acid-alcohol, embedded in celloidin, sectioned, and stained with Delafield haematoxylin-eosin and with Orseillin BB. A series of sections, usually 10 μ in thickness, was cut from cankers collected throughout the fall, winter, and spring.

All the cankers are walled off from the healthy cortex by a layer of wound cork 5 to 10 cells deep. The fungus seldom penetrates very deeply into the

cortex and remains between the cells at all times. The intercellular mycelium appears always to be made up of short cells, and it is not certain just how the pathogen obtains its nutrition from the host cells. The sections do not show that the fungus possesses haustoria or extends beyond the layers of cork tissue. These observations explain why the cankers are generally superficial and are sloughed off as the twigs and branches become older.

In cankers collected during October, November, and December mycelium is present between the cells of the host, but no acervuli are formed. During January, February, and March changes take place that lead to the formation of the acervuli.

The acervulus is formed beneath the epidermis of the host by an apparent massing of mycelial strands growing out from between the cells of the host. As the acervulus increases in size, pressure is exerted on the epidermis of the host, forming blisters that are visible to the naked eye. As the acervulus matures, the characteristic conidia are formed beneath the epidermis of the host. A few of the acervuli bear conidia in March, but during April and May the great majority of them mature and the conidia are exposed by a breaking away of the thin epidermal layer of the host.

Supplemental to the collection and fixation of cankered material during the winter and early spring of 1935–36, pure culture isolations were made from similar material at various times. In nearly all cases the pathogen was isolated without difficulty.

In 1936 and 1937 similar experiments were conducted at Springfield and Mountain Grove, Mo., to find out whether or not bark cankers are infection sources in the spring. Trees pruned of their 1935 wood and not exposed to overwintered diseased leaves were caged with muslin. Trees not pruned of their 1935 wood and not exposed to overwintered diseased leaves were also caged with muslin. Noncaged trees, pruned and not pruned of their 1935 wood, were exposed to overwintered diseased leaves, and similar trees were

TABLE 2.—*Leaf infection from conidia produced from overwintered cankers on pear twigs and watersprouts at Mountain Grove, and Springfield, Missouri*

No. of trees	Treatment	Date of first observed infection in 1936	Period of maximum infection in 1936
2	Caged, pruned. Overwintered leaves removed	Not infected	.....
2	Caged, nonpruned. Ooverwintered leaves removed	May 7–8	June 10–20
4	Noncaged, nonpruned. Overwintered leaves left at base of trees	May 7–10	June 8–16
4	Noncaged, nonpruned. Leaves removed from base of trees. Ground scraped	May 9–12	June 10–15
4	Noncaged, pruned. Overwintered leaves left at base of trees	May 20–21	June 10–15
4	Noncaged, pruned. Leaves removed from base of trees. Ground scraped	May 27–June 1	June 10–15

not exposed to overwintered diseased leaves. The results of these experiments are shown in table 2. The experimental trees were known to have been heavily cankered in 1935, but nothing was known about their 1934 disease history. It is clearly shown that the removal of the 1935 bark cankers was responsible for a lack of the disease where the tree was protected by muslin from chance infections. It also is clearly shown that trees deprived of their diseased overwintered leaves, but not deprived of their 1935 cankers, became infected as early as those not deprived of their overwintered diseased leaves and 1935 wood. This indicates again that overwintered diseased leaves are not the overwintering agency. Noncaged trees deprived of their 1935 wood, either with or without the overwintered diseased leaves left at their bases, became infected much later than those exposed to overwintering bark cankers. These probably were chance infections and clearly indicate again the importance of the 1935 wood in causing the early infections. The same results were obtained at Mountain Grove and at Springfield, Mo., but the dates of infection differed slightly between the two stations. This accounts for the two dates appearing in the second column of table 2.

In 1937 two of the trees used in the 1936 experiments were studied further to find out whether or not the bark cankers formed during 1935 were still active during the 1937 season. From one of these all the 1935 and 1936 wood was removed; from another, all the 1936 wood was removed. Both trees were caged and all the leaves were removed from beneath both of them.

During the 1937 season, foliage infection, slight to begin with, developed on the tree deprived of its 1936 wood but not of its 1935 wood. No infection developed on the tree deprived of its 1935 and 1936 wood. This apparently indicated that the organism overwintered the second year in cankers on the 1935 wood. Table 2 indicates that this was not so in the case of cankers studied during 1935. Since we have observed that conidia remain in cankers during a great part of the season and also occasionally during the early part of the next season, it may be possible that chance infections may arise from this source during the second season after the cankers are formed. We have observed several times that slight infections develop in trees that have previously been kept clean for a season. It is possible that these infections arise from conidia that overwinter in the old established cankers. If the mycelium overwintered during the second winter in these old cankers, one would expect that the initial infections that arise from these sources the following spring would be of a major character. Since the infections are always slight, it is believed that only previously formed conidia survived the second season.

Spore trapping experiments also were conducted for the purpose of finding out when and under what conditions conidia were being disseminated from the bark cankers. It was found that, starting soon after the middle of April and continuing as late as the first week in June, 1936, conidia were being washed from the mature bark-canker acervuli during each rainy period. The greatest dissemination occurred during late May and early June, but a few mature conidia could be found in the cankers very late in the summer

and in the following spring. At the same time, in 1936, spore-trapping experiments with diseased overwintered leaves showed neither ascospores nor conidia to be present. During the spring of 1937 asci were absent but conidia were common on overwintered diseased leaves. This may be attributed to the mild winter of 1936–37.

SUMMARY

Leaf blight of pear and quince, caused by *Fabraea maculata* (Lév.) Atk., is a common disease in the middle-western and eastern parts of the United States and has been reported from many of our States. It is also common in many countries and attacks various pomaceous hosts.

While the ascospore stage has been observed infrequently by others, it has not been found on overwintered pear leaves under Maryland and Missouri conditions.

In Maryland and Missouri, conidia have been found to survive the winter on diseased leaves but not on diseased fruits. Occasionally, they overwinter in bark cankers.

Experiments have shown that overwintered conidia on diseased leaves may cause spring infections, but the evidence accumulated during several years indicates that these are not of importance in spreading the disease.

In Maryland and Missouri it has been found that the leaf-blight pathogen overwinters principally as mycelium in cankers formed in the bark.

The mycelium in the overwintering cankers forms acervuli during the late winter months, and, during early spring, conidia are produced in the acervuli and, escaping through cracks in the bark, infect the unfolding leaves.

Spore trapping experiments show that the conidia which are produced in the spring are disseminated from the cankers over a period of several months.

Conidia, escaping from the bark cankers, are washed downward through the tree, and cause in the leaves beneath the cankers cone-shape tracts of infection.

Conidia escaping from the leaf lesions may cause infections on the young bark of the growing stems any time during the growing season. In some of these bark infections the mycelium overwinters, and the life cycle of the pathogen is completed when conidia are produced in them the following spring.

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FURTHER STUDIES OF CRINKLE LEAF, A DISORDER OF COTTON PLANTS PREVALENT IN LINTONIA AND OLIVIER SILT LOAM SOILS OF LOUISIANA

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INTRODUCTION

In a previous publication[3] crinkle leaf, a new disease of cotton in Louisiana was described. It was pointed out that the disease was first observed in Louisiana in 1934 in certain areas of Lintonia and Olivier silt loam soils, and that in 1937 it was observed again in 4 additional fields in East Baton Rouge Parish, the disease occurring in a severe form in one of these fields with damage estimated at about 10 per cent.

It was further shown that the disease occurred on several varieties of cotton, that it is not hereditary, and that attempts to transmit it to healthy plants by grafting and budding and by injecting sap collected from various parts of affected plants were unsuccessful. Since the above-mentioned inoculation experiments demonstrated fairly conclusively that crinkle leaf is not a virosis, notwithstanding the similarity of some of the symptoms to those of viroses of other plants, and it was decided to make a study of certain soil factors—toxic substances or deficiencies that might be responsible for the disease. In preliminary experiments previous cultural and fertilizer practices of affected areas were studied and the pH and water-soluble manganese

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[3] Neal, D. C. Crinkle leaf, a new disease of cotton in Louisiana. Phytopath. **27**: 1171–1175. 1937.

of the soil of affected and nonaffected areas were determined. Later, in both field and pot experiments, nutritional and toxicity studies also were made in an attempt to control and to induce the disease. These observations and experiments form the basis of this report.

PREVIOUS CULTURAL AND FERTILIZER TREATMENT OF AFFECTED AREAS

At the old experiment station in Baton Rouge, where the malady was first observed by H. B. Brown in 1934, the soil had been in cultivation for many years and had been fertilized for the 1934 cotton planting with 200 pounds of nitrate of soda, 300 pounds of superphosphate, and 50 pounds of muriate of potash per acre. At Pride, Louisiana, where the disease appeared in a severe form in 1937, the soil also had been in cultivation many years and had received for the 1937 crop 400 pounds per acre of a commercially mixed 4–12–4 fertilizer in addition to a ton per acre of barnyard manure. A striking feature of the soil of affected areas during the growing season was observed. It exhibits a moist, brownish surface discoloration after being exposed to rain and aeration for several days, giving the appearance of having received a large application of nitrates or some other deliquescent salt. This condition was lacking in areas where the cotton grew normally.

THE pH, WATER-SOLUBLE MANGANESE, AND WATER-SOLUBLE SALTS OF AFFECTED AND UNAFFECTED AREAS, AND TYPE OF PLANT GROWTH FOLLOWING CERTAIN SOIL TREATMENTS

A comparison of the pH of affected and unaffected areas showed without exception that the soil of the affected area had a lower pH. A comparison was then made of the water-soluble manganese and water-soluble salts and it was found that soil from the affected areas yielded approximately 4 times as much manganese as was obtained from the unaffected. More water-soluble salts and nitrates also were found in the affected areas (Table 1).

TABLE 1.—*Effect of different treatments of soil on the development of crinkle leaf in the field and in pot cultures*

Soil from	Treatment	pH	H_2O soluble Mn.	H_2O soluble salts	NO_3 N	Type of plant growth
			p.p.m.	*p.p.m.*	*p.p.m.*	
Affected field	400 lbs. 4–12–4 1 ton barnyard manure	5.0	46.4	580	90.9	Crinkle-leaf plants or none at all
Unaffected field	Same	5.5	11.9	440	55.5	Normal plants
Affected field in pots[a]	Same	5.1	52.3	.....	.....	Crinkle-leaf plants
Affected field in pots	Same plus 1 ton $CaCO_3$	6.4	0.8	.....	.....	Normal, healthy plants

[a] 8 kilos per pot.

FIELD AND POT EXPERIMENTS

The preliminary determinations of the pH and soluble manganese of the soil from the affected areas indicated that these factors were closely correlated with the disease. Therefore, a number of treatments were made on June 21 at Pride, Louisiana, to single affected Deltapine cotton plants in the field for an indication of a suitable control. Included in these preliminary tests were:

Treatment	Rate per acre lbs.
Calcium nitrate	200
Superphosphate	400
Potassium chloride	100
Sulphate of potash—magnesia	100
Calcium sulphate	1500
Calcium carbonate	1500
Calcium carbonate plus N.P.K.	1500
Copper, zinc, and boron solution to yield 1 p.p.m. each.	

Subsequent readings made later in the season showed that calcium carbonate and calcium carbonate plus N, P, and K gave some recovery. Later treatments, where the cotton was planted July 20 in 5-foot-row sections, showed that 3000 pounds of calcium carbonate broadcast and 500 pounds in the drill gave healthy seedlings.

With this indication, a series of pot-culture experiments were set up in which a better study could be made on seedlings. The results showed that calcium, magnesium, and potassium carbonates, equivalent to a rate of 1000 pounds per acre or more of calcium carbonate, were correctives for the disease (Fig. 1), the plants remaining healthy for a period of approximately 60 days, while the plants grown in nontreated soil from an affected area readily developed crinkle-leaf symptoms. Calcium sulphate, at the rate of 1000 pounds per acre in pot experiments, gave no control of the disease.

As a result of the above investigation it was apparent that the hydrogen-ion concentration or some substance made soluble by the high acidity was responsible for the disease. To test this possibility a series of pots was set up with river-washed sand[4] planted in duplicate to Deltapine cotton. All pots were adjusted to pH 5.0, including the controls. After the seedlings had formed true leaves, increasing amounts of manganous sulphate were added to some of the pots, 0.12 to 0.16 grams manganese per pot being the two highest increments, and increasing amounts of aluminum sulphate to the remainder. Hartwell and Pember's solution was added at intervals as needed. It was soon evident that insufficient amounts of manganese and aluminum had been added, so the amounts of manganese were increased to 0.2 and 0.5 grams manganese per pot for the 2 highest increments. What appeared to be the symptoms of crinkle leaf began to develop in about 22 days in plants that received the second treatment of manganese. The plants in the pots receiving moderate to large amounts of aluminum (0.08 to 0.32 grams per pot) remained normal or died without showing any symptoms of

[4] Each pot contained 7 kilograms.

FIG. 1. Deltapine cotton plants growing in Olivier silt loam. Left, soil from an area affected with crinkle leaf and treated with 50 pounds per acre of manganese sulphate; center, soil from an affected area, nontreated; right, soil from an affected area and treated with 1000 pounds per acre of calcium carbonate. Plants photographed after 60 days' growth.

TABLE 2.—*Effect of treating cotton seedlings in sand culture with manganese*

Sand culture 7 kilos per pot	Treatment[a] per pot	pH	H_2O[b] soluble Mn recovered	Plant growth after 25 days
			p.p.m.	
1	No Mn	5.1	None	Normal, healthy plants
4	0.3 gram Mn[c]	5.0	27.2	Slightly chlorotic showing leaf-margin injury
8	0.7 gram Mn	5.0	41.7	Chlorotic, crinkling, flecking and retarded growth
9	1.0 gram Mn	5.2	74.8	Chlorotic, crinkling, flecking and retarded growth

[a] Hartwell and Pember's nutrient solution was added as needed to maintain rapid growth.

[b] Basis oven-dry sand.

[c] Supplied from manganous sulphate.

the disease. The concentration of the manganese finally was increased to 0.3 and 0.6 grams per pot in the 2 highest treatments originally used, and a fair reproduction of the symptoms was obtained. However, the affected plants were not dwarfed, due, no doubt, to the late application of sufficient manganese. Finally, a second set of sand cultures was set up and planted. At this planting a range of concentrations of manganese were added that was expected to cover the point at which typical symptoms could be obtained. All pots were again adjusted to pH 5.0, including the controls. The results obtained, together with the concentrations of manganese employed, are shown in table 2. In this experiment symptoms of crinkle leaf appeared in 12 days from the date of planting and within 25 days typical symptoms of the disease were produced (Fig. 2).

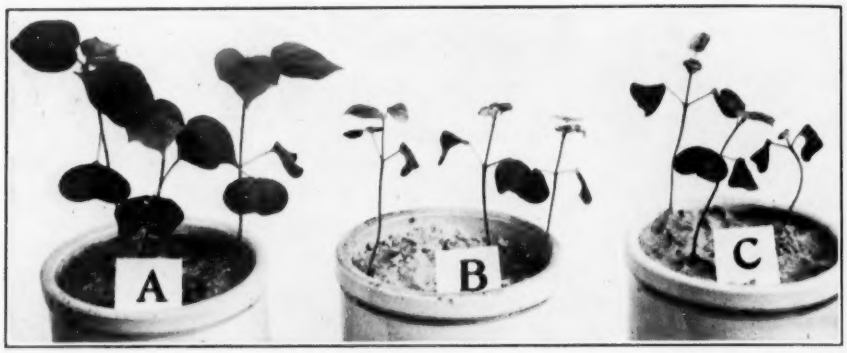

FIG. 2. Deltapine cotton plants grown in sand cultures with Hartwell and Pember's solution and varying amounts of manganese. A. Full nutrient solution only. B. Full nutrient solution plus 0.6 gram manganese. C. Full nutrient solution plus 1 gram manganese. Photographed after 25 days' growth.

DISCUSSION AND SUMMARY

As a result of experiments herein reported, it is indicated that the disease of cotton known as crinkle leaf and prevalent in certain Lintonia and Olivier silt-loam soils in Louisiana is associated with high acidity, calcium deficiency, and manganese toxicity.

Although the disease is readily corrected by calcium and other basic carbonates, it is not caused by a deficiency of calcium *per se*. In pot and field experiments calcium was supplied in the form of calcium sulphate in amounts sufficient for nutritional requirements, yet it had no effect in controlling the disease. This, very probably, is because of the fact that the hydrogen-ion concentration of the soil in the pots treated with the calcium sulphate remained essentially unchanged (pH 4.8 to 5), thus allowing manganese to remain in solution and cause the toxic effect as described for crinkle leaf. At the higher pH produced by treating the soil with the basic carbonates, calcium, magnesium, and potassium carbonate, the manganese apparently is precipitated and, accordingly, the plants remain healthy. The further evidence that soil from areas in Louisiana affected with crinkle leaf have a

consistently lower pH and contain significantly larger amounts of manganese than soil from healthy areas indicates that the disease is attributable to manganese toxicity. Moreover, reproduction of its typical symptoms in cotton plants in sand cultures by the addition of increasing amounts of manganese sulphate, as reported herein, strengthens this viewpoint.

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PATHOGENICITY OF CULTURE-REARED SPECIMENS OF THE BUD-AND-LEAF NEMATODE AND THE SUSCEPTIBILITY OF DIFFERENT STRAWBERRY VARIETIES

J. R. CHRISTIE

(Accepted for publication April 25, 1938)

THE PATHOGENICITY OF CULTURE-REARED SPECIMENS

In a former paper[1] it was pointed out that the southern strawberry strain of *Aphelenchoides fragariae* (Ritzema Bos, 1891) can be successfully reared on agar cultures. Before using this procedure in the investigation of summer dwarf it was deemed advisable to determine if these culture-reared specimens, when transferred to strawberry plants, would produce typical symptoms of the disease.

In the following tests, unless otherwise noted, the specimens used to inoculate the plants were washed from the cultures with water, concentrated in a small amount of fluid by centrifuging and transferred to the crown of the plant with a dropper. In all cases the nematode population employed had been on culture continuously since September 13, 1935. Strawberry plants of the variety Blakemore were used throughout.

Greenhouse Experiment.—In this test, conducted in a greenhouse at Washington, D. C., 45 potted strawberry plants were arranged in 3 lots of 15 plants each, which, for convenience, are designated A, B, and C. Lots A and C were inoculated with specimens of *Aphelenchoides fragariae* direct from the cultures. Lot B was inoculated with culture-reared specimens that were surface-sterilized for 30 minutes in a 1 to 2000 solution of mercuric chloride. The method of sterilizing was the same as that given in a former paper.[1]

Lots A and B were inoculated May 22, 1936. Disease symptoms appeared in about 6 weeks and the plants were examined microscopically between July 30 and August 3, or after 69 to 73 days. The plants in lot C, inoculated July 17, began to show symptoms in about 4 weeks and were examined microscopically on August 20, or after 34 days. The results of this experiment are shown in table 1. Forty noninfested plants served as controls, 10 of

[1] Christie, J. R., and Louise Crossman. Notes on the strawberry strains of the bud and leaf nematode, *Aphelenchoides fragariae*, I. Helminthol. Soc. Washington Proc. 3: 69–72. 1936.

which were examined microscopically on August 11. None of the control plants developed recognizable symptoms, and specimens of *Aphelenchoides fragariae* were not found in those examined. In all 3 lots symptoms were moderately pronounced (Fig. 1). The plants in lot B that were inoculated

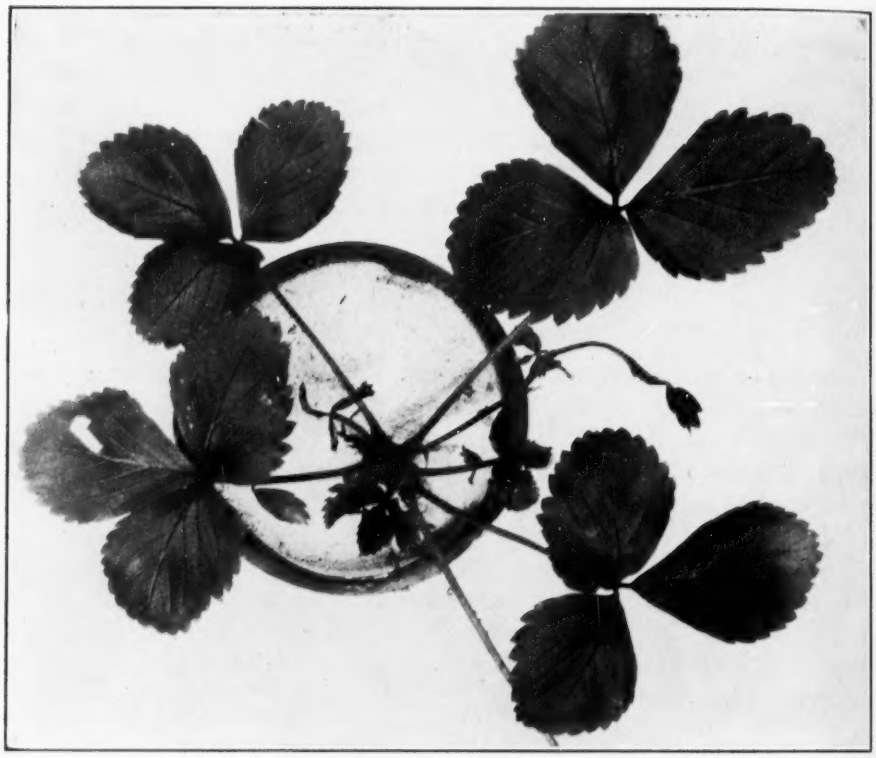

FIG. 1. Typical symptoms of summer dwarf on strawberry plant inoculated with culture-reared specimens of *Aphelenchoides fragariae* in a greenhouse.

with surface-sterilized specimens showed no appreciable difference from the others.

TABLE 1.—*Development of symptoms of summer dwarf on strawberry plants inoculated with culture-reared specimens of Aphelenchoides fragariae and grown in the greenhouse*

Condition of plants	Lot A		Lot B		Lot C	
	No. infested	No. not infested	No. infested	No. not infested	No. infested	No. not infested
With unquestionable symptoms	12	0	11	0	10	0
With possible symptoms	0	0	0	1	2	1
Normal	0	3	3	0	2	0

Field Experiment.—This test was conducted in the open field at the North Carolina Coastal Plain Station, Willard, N. C. On January 13, 1936, 78

strawberry plants were set in 3 rows of 26 plants each, which, for convenience, are designated A, B, and C. For row A the nematodes were washed from the cultures with water and concentrated in a small amount of fluid by centrifuging. After sundown on July 22, about 2 or 3 drops of this fluid were placed in the crown of each plant. For row C the nematodes were washed from the cultures with water but were not concentrated by centrifuging. During the early forenoon of July 23 about 6 or 8 cc. of this water were placed in the crown of each plant. A considerable part of the fluid ran into the soil at the base of the plant. The plants in row B (the middle row) were not inoculated and served as controls. Data were secured on August 25, September 21, and October 20 and are summarized in table 2.

TABLE 2.—*Development of symptoms of summer dwarf on strawberry plants inoculated with culture-reared specimens of Aphelenchoides fragariae and grown in the open field*

Condition of plants	No. of plants Row A			No. of plants Row B			No. of plants Row C		
	Aug. 25	Sept. 21	Oct. 20	Aug. 25	Sept. 21	Oct. 20	Aug. 25	Sept. 21	Oct. 20
With very pronounced symptoms	9	9	15	.....	.....	1	.....	.....	9
With moderate but unquestionable symptoms	8	14	8	.....	.....	1	3	18	12
With possible symptoms	4	.....	.....	.....	.....	2	2	4	2
Normal	4	1	1	25	25	21	21	3	1
Dead	1	2	2	1	1	1	.....	1	2

In general, symptoms were much more severe than in the greenhouse test. Although the rows were only about 3 feet apart, it was not until October 20 that symptoms appeared in row B, presumably through spread from the adjoining rows. The plants were not examined microscopically.

The nematodes used in the 2 preceding experiments had been maintained continuously on cultures for from 8 to 10 months, and those used in the experiment discussed in the following section had been maintained on culture for 21 months. These experiments appear to demonstrate that rearing this strain of *Aphelenchoides fragariae* on culture does not reduce its pathogenicity to strawberry plants.

RELATIVE TOLERANCE OF DIFFERENT STRAWBERRY VARIETIES TO SUMMER DWARF

The following experiment was conducted at the North Carolina Coastal Plain Station, Willard, N. C. A plot consisting of 20 plants each of 36 different varieties of cultivated strawberries was established. The plants were secured from a dealer in Maryland and were transplanted on March 24, 1937. They were inoculated with culture-reared specimens of the southern strawberry strain of *Aphelenchoides fragariae* on June 23, 1937. The specimens were washed from the cultures with water, and concentrated in a small

amount of fluid by centrifuging. Three or 4 drops of this fluid were placed in the crown of each plant. Apparently, every plant had an equal opportunity to become infested. The nematode population was the same as that used in the experiments already discussed and had been maintained on culture continuously for 21 months. The data given in table 3 were secured on August 7, 1937.

TABLE 3.—*Development of symptoms of summer dwarf in different varieties of cultivated strawberries. Based on 20 plants of each variety*

Variety	Unquestionable symptoms	Possible symptoms	Dead
Aberdeen	6	4	6
Aroma	4	4	12
Beauty	11	3	6
Bellmar	7	2	4
Big Joe	8	–	9
Big Late	5	3	12
Blakemore	18	–	1
Catskill	8	–	7
Champion (= Progressive)[a]	5	–	11
Chesapeake	5	–	14
Clermont	9	–	9
Culver	1	3	3
Dorsett	16	2	2
Dr. Burrell (= Senator Dunlap)	7	–	11
Fairfax	13	3	3
Gandy	7	1	12
Gem[a]	1	–	17
Gibson (= Parson's Beauty)	8	2	9
Glen Mary	11	–	9
Green Mountain[a]	3	3	10
Haverland	2	–	18
Howard 17 (= Premier)	15	2	1
Klondike	12	1	5
Lupton	9	–	11
Marshall	6	2	10
Mastodon[a]	12	2	3
Missionary	14	1	3
Narcissa	12	–	4
New York	17	–	1
Orem	7	2	11
Pearl	6	1	16
Parson's Beauty (= Gibson)	5	–	13
Premier (= Howard 17)	11	1	4
Progressive (= Champion)[a]	1	3	15
Redheart	1	3	14
Sample	7	–	13
Senator Dunlap (= Dr. Burrell)	6	–	13
Southland	1	3	9
Wayzata or Rockhill[a]	7	–	11
Wm. Belt	8	3	5

[a] "Everbearing" varieties.

Some of the varieties of strawberries used in the experiment are known by more than one name. In several instances 2 bundles of plants were received from the grower, each under a different name, although, strictly speaking, both were the same variety. These were set in the plot under separate label.

Many of the varieties tested are not adapted to the climatic conditions of North Carolina. When final observations were made, November 7, 1937, the surviving plants of the varieties Haverland, Gem, Progressive, Redheart, Aroma, Green Mountain, and Wayzata were very small and weak. Lack of vigor was noticeable also in the varieties Chesapeake, Senator Dunlap, Parson's Beauty, Sample, and, to a less extent, in others. Failure of the plants of some of these varieties to survive and the poor growth of those that did survive is largely attributable to unfavorable climatic conditions.

The experiment demonstrated that all the varieties tested may become infested with the southern strawberry strain of *Aphelenchoides fragariae* and, when infested, may develop symptoms. The relative severity of symptoms varied considerably with different varieties. Glen Mary was the most severely affected, all the surviving plants showing exceedingly pronounced symptoms. Blakemore, Chesapeake, and Lupton also developed especially severe symptoms. Other varieties that developed severe symptoms, but perhaps not quite so severe as those already mentioned, were Bellmar, Catskill, Clermont, Dorsett, Fairfax, Klondike, Missionary, Narcissa, New York, and Premier.

Of the varieties on which symptoms were slight, the most outstanding was Beauty. Fourteen plants of this variety were obviously affected but all made a relatively vigorous growth and were not dwarfed, the abnormality consisting of distorted and crinkled leaves. Aberdeen, Culver, and Southland were other varieties on which symptoms did not appear to be very severe, but with these, especially the last two mentioned, failure to become infested may be the explanation. Symptoms on Mastodon were only moderately severe.

On the other varieties not mentioned above symptoms varied somewhat but, in general, seemed moderately severe. In some instances, as already noted, so few plants survived or those that did survive made such a weak growth that a reliable appraisal of the severity of symptoms was difficult.

SUMMARY AND CONCLUSIONS

When strawberry plants were inoculated with specimens of the southern strawberry strain of *Aphelenchoides fragariae* from agar cultures that had been maintained continuously for from 8 to 21 months, typical symptoms of summer dwarf usually resulted, both in the greenhouse at Washington, D. C., and in the open field at Willard, N. C. It is concluded that one may use culture-reared specimens of this nematode in experimental work with every assurance of securing the same results as would be secured were the nematodes obtained directly from the plants.

A plot composed of 20 plants each of 38 different varieties of cultivated strawberries was established at Willard, N. C. When inoculated with culture-reared specimens of the southern strawberry strain of *A. fragariae*, one or more plants of every variety developed typical symptoms of summer dwarf, but the severity of the symptoms varied considerably in different varieties. This is probably a matter of tolerance, and does not appear to be entirely correlated with vigor of growth.

SUGGESTED ROLE OF ALKALOIDS IN PLANTS RESISTANT TO PHYMATOTRICHUM OMNIVORUM

GLENN A. GREATHOUSE

(Accepted for publication, April 9, 1938)

Taubenhaus and Ezekiel[1] have reported observations on more than 2,000 species or varieties of plants with regard to their relative resistance to *Phymatotrichum omnivorum* (Shear) Duggar. Of these approximately 19 per cent were found to be immune from the disease. A great part of the remainder were quite susceptible, although some species were resistant during most or part of their development.

Preliminary studies indicated the presence of alkaloids in crude extracts from roots of several plants resistant to *P. omnivorum*. A number of these complex substances have been isolated and shown to inhibit the growth of the fungus in the same or even lower concentration than that found in the plant tissue. To test further the possible rôle of alkaloids as inhibitors of the growth of the above-named organism, a survey of the literature was made for the purpose of noting possible correlations between the presence or absence of alkaloids and the classification of plants for resistance to *P. omnivorum*. Alkaloid-containing plants that have not been tested or observed for reaction to the root-rot organism were not included, although a considerable number of them are closely related to plants or groups known to be resistant or immune. The results of this study are set forth in table 1.

TABLE 1.—*Grouping of varieties and species of plants according to alkaloid content and their resistance or susceptibility to Phymatotrichum omnivorum*

Rating of plants as to disease reaction	Total	Extremely susceptible	Highly susceptible	Moderately susceptible	Resistant	Immune
	2116	54	375	876	403	408
Species in which alkaloids have been reported[a]	125	0	2	40	44	39
As percentage of the different groups			0.55	4.56	10.92	9.56

[a] Compiled from numerous publications on alkaloids.

Table 1 indicates a direct relationship between the presence of alkaloids in plants and their relative resistance to the Phymatotrichum root-rot organism. The percentages of the different groups are 0, 0.55, 4.56, 10.92, and 9.56, respectively, for the extremely susceptible to the immune group. It can be noted that 83 of the 125 plants that contain alkaloids fall within the resistant and immune groups.

[1] Taubenhaus, J. J. and W. N. Ezekiel. A rating of plants with reference to their relative resistance or susceptibility of Phymatotrichum root-rot. Texas Agricultural Experiment Station Bulletin 527. 1936.

Salgues[2] found that *Colchicum autumnale* bulbs, attacked by *Urocystis* (*Tuburcinia*) *colchici*, contained only 0.044 per cent colchicine as against 0.115 per cent in those of healthy plants.

Schmidt,[3] and Agerberg *et al.*,[4] have shown that solanine caused abnormal type of growth in *Cladosporium fulvum*.

Nobécourt[5] suggested that alkaloids may protect plants against the attack of parasitic fungi. To test this hypothesis *Botrytis cinerea* was grown in Raulins solution to which different amounts of alkaloids were added. He studied the effect of only a few alkaloids and concluded that only one of the alkaloids used might occur in a plant in sufficient concentration to serve as a protection against this fungus.

The above studies, although of limited scope, and involving other diseases, tend to support the correlation indicated in table 1, and lend further support to the writer's proposal that the presence of alkaloids may partially explain the resistance of certain plants to Phymatotrichum root rot. It is not suggested that the mere presence of an alkaloid in a host plant necessarily warrants the conclusion that it plays a rôle in the resistance of that host to a given parasite or parasites, since recent work in our laboratory has shown that alkaloids with different nuclei or arrangement within a nucleus possess different inhibitory values. Furthermore, alkaloids may be present in concentrations so low that their inhibitory effects are negligible, or even stimulative to the organism.

Frequently more than one alkaloid is present in a given tissue. Therefore it is not essential that every alkaloid in the tissue be equally inhibitive in order to ward off or restrict the attack of the pathogenic organism. This fact has been confirmed by laboratory studies.

The relationship between the presence of alkaloids in the tissues of plants and the resistance of these plants to *P. omnivorum* is receiving intensive study. Meanwhile it was thought that this note might be of interest to other students of disease resistance since the literature on this subject is limited.

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SUSCEPTIBILITY OF CROP PLANTS AND WEEDS TO SCLEROTIUM ROLFSII

J. S. Cooley

(Accepted for publication April 23, 1938)

Southern blight, caused by *Sclerotium rolfsii*, has been known since 1922 as a disease of apple stocks in South Africa.[1] It was reported as a nursery disease of apple trees in Australia in 1933,[2] and in 1936,[3] as a nursery disease of apple trees in this country.

In August, 1935, *Sclerotium rolfsii* Sacc., was observed in an apple-tree nursery at the U. S. Horticultural Field Station, Beltsville, Maryland. Again, in 1936, the fungus was in evidence and more trees, then 2 years old, were killed by it. After the trees were removed from the nursery in the spring of 1937, a plot where 20 per cent of the apple trees were killed in 1935 and 1936 was planted with 12 vegetable and forage crops to determine their susceptibility to the disease. The rows were run at right angles to those of the nursery in the previous planting.

The plants grown in this plot were pepper, tomato, eggplant, cowpeas, soy beans, navy beans, Kidney beans, red clover, Mammoth clover, alsike clover, spring oats, and Korean lespedeza, *Lespedeza stipulacea* Maxim. The seed was planted or the plants set on May 18. After about 3 weeks, 1 of 30 pepper plants, 1 Kidney bean and 2 cowpea plants out of several hundred were dead from the disease. After 4 months, 2 navy bean and 2 more cowpea plants were dead, but none of the clovers. On July 29, or 8½ weeks after planting, there was no further advance of the disease on the beans or peppers, but the lespedeza was severely affected. The disease seemed to have started at one or two points and advanced along the row till about 20 per cent of the plants were affected. On August 20, or 13½ weeks after planting, additional Korean lespedeza plants were killed, but there was no more advance on the other kinds of plants. By the end of the season, all the lespedeza plants were killed except those in a 6-foot section of a 30-foot row.

During the wet weather the latter part of the summer, most of the clover plants died, but the sclerotium disease was not apparent on them. This malady has been reported attacking clover, but, under these conditions, these 3 varieties apparently were unaffected. The remainder of the block, in which the disease was present in 1936 and was not used for this experiment, was planted to Wilson soy beans. They showed no apparent indication of injury because of the disease.

In July, cultivation was discontinued and weeds and wild grasses grew

[1] Birmingham, W. A. Sclerotium disease of Northern Spy stocks. Jour. Dept. Agr., Union South Africa **4**: 405. 1922.

[2] Birmingham, W. A. Another fungus attacking apple stocks. Agr. Gaz., N. S. W., **44**: 58–60. 1933.

[3] Cooley, J. S. *Sclerotium rolfsii* as a disease of nursery apple trees. Phytopath. **26**: 1081–1083. 1936.

in the plot. In August, the following plants were noted as unaffected by the disease, even when growing in close proximity to diseased lespedeza: Pigweed, *Amaranthus retroflexus* L.; 3-seeded mercury, *Acalypha virginica* L.; Cuphea, *Cuphea petiolata* L.; ragweed, *Ambrosia artemisiifolia* L.; and foxtail grass, *Setaria glauca* L. Plants showing slight infection were spurge, *Euphorbia preslii* Guss. and common purslane, *Portulaca oleracea* L.; but the advance was not rapid nor was a large percentage of the plants affected. Since healthy plants, even of these two partially susceptible species, were growing about diseased lespedeza plants, it seems likely that, under these conditions, none of the weeds reported above are very susceptible to *Sclerotium rolfsii*. The fact that most of the other plants used, which are recorded in the literature as being susceptible to the disease in other regions, were so slightly affected under these conditions, may be because conditions were so unfavorable that only a few plants took the disease. Since this sclerotium disease heretofore has not been known to be serious as far north as this region, it seems very likely that climatic conditions are usually unfavorable here for its propagation and maintenance. Unfavorable conditions for the overwintering and infection of the disease in 1938 rather than lack of epidemic and abundance of sclerotia in 1937 probably account for the slight infection on plants that are reported in the literature as moderately susceptible. Even the highly susceptible lespedeza, which eventually was nearly all killed by the disease, showed initial infection at only 2 points in the row and spread from these foci of infection, killing all the plants as far as it spread. These observations point to the conclusion that, under these conditions, relatively few viable sclerotia survived the winter.

The results of this one year's experiment indicate that Korean lespedeza may be sufficiently susceptible to be useful as an indicator crop to determine whether or not a given plot contains the sclerotium disease. This experiment indicates that Korean lespedeza should not be used as a cover crop preparatory to planting apple nursery stock or other susceptible plants.

U. S. HORTICULTURAL FIELD STATION,
BELTSVILLE, MARYLAND.

PHYTOPATHOLOGICAL NOTES

Pythium irregulare and Damping off of Watermelons.[1]—Watermelon plants, whether inherently resistant or susceptible to *Fusarium bulbigenum* Cke. and Mass. var. *niveum* (E.F.S.) Wr., often damp off and wilt during the early seedling stage. This seedling loss has commonly been attributed to the wilt pathogen, but studies made recently indicate that certain other soil-inhabiting pathogens are partially responsible.

Fifteen isolations were made in the spring of 1937 at Conesville, Iowa, from field-grown, wilt-resistant, watermelon seedlings displaying wilted cotyledons, water-soaked hypocotyls, and, in some instances, parasitized roots. Thirteen of the fungi recovered were *Pythium* sp. One of the 2 remaining fungi was *Fusarium bulbigenum* var. *niveum*, and the other was an unidentified saprophyte. Eleven of the 13 *Pythium* isolates were tentatively assigned to *P. irregulare* Buisman, while the other 2 were not identified.

The pathogenicity of the different isolates of *Pythium irregulare* was demonstrated in 4 separate tests on artificially infested, autoclaved soil. In each of the 4 tests, 11 greenhouse flats contained infested soil were used, while 2 flats containing autoclaved soil were used as checks. Thirty surface-disinfected seeds of a wilt-resistant variety (Improved Stone Mountain No. 5) and a wilt-susceptible variety (Dixie Queen) were planted in each flat. The planted flats were held in a greenhouse where the temperature remained near 20° C. It was found that *P. irregulare* caused seed decay, preemergence and postemergence damping off, and root necrosis of the seedlings, regardless of their resistance or susceptibility to the wilt pathogen. The average stand of seedlings of the wilt-resistant variety, in artificially infested soil, was 37.3 per cent (30 days after planting), while that of the wilt-susceptible variety was 40.3 per cent. Average seedling stands of the 2 varieties, when planted in autoclaved soil, were 93.8 and 97.2 per cent, respectively. The pathogen was isolated repeatedly from infected roots, hypocotyls, and cotyledons of plants grown in infested soil, and also from seeds that had failed to germinate in such soil.

Fusarium bulbigenum var. *niveum* and *Pythium irregulare* produced similar damping-off symptoms under controlled conditions. The pathogens were capable of attacking roots, hypocotyls, and cotyledons of seedlings and of causing general necrosis. It is believed that seedling losses under field conditions are not entirely attributable to *F. bulbigenum* var. *niveum*, but that *P. irregulare* may be responsible for a portion of the damage.—S. G. YOUNKIN, Iowa Agricultural Experiment Station, Ames, Iowa.

[1] Journal Paper No. J-552 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 71.

Where Are Gardenia Cankers Initiated?[1]—Several investigators[2, 3, 4, 5, 6, 7] have found that *Phomopsis gardeniae* Hansen and Barrett, the cause of gardenia canker and gall, is a weak parasite. They have emphasized that the fungus enters the host through wounds made either by insects or during cultivation.

FIG. 1. Twelve-week-old rooted *Gardenia florida* var. *veitchii* cuttings. Left: cankers at three nodes where leaves had been removed prior to embedding the cuttings in the propagating bench. Right: a more extensive canker.

[1] Journal series paper.

[2] Hansen, H. N., and C. E. Scott. A canker and gall disease of gardenia. Science (n.s.) **79**: 18. 1933.

[3] ———, and J. T. Barrett. Gardenia canker. Mycologia **30**: 15–19. 1938.

[4] Huber, G. A. A Phomopsis canker and gall disease of gardenia. Flor. Exch. and Hort. Tr. World **86**: 11. 1936.

[5] Obee, D. J. A note on the canker disease of gardenias. Trans. Kans. Acad. Sci. (1936) **39**: 103–104. 1937.

[6] Tilford, P. E. Stem canker disease of gardenia. Ohio Agr. Exp. Sta. Bimonth. Bull. **19**: 116–117. 1934.

[7] White, H. E. Careful culture recommended to avoid canker on gardenia. Flor. Rev. **79**: 9–10. 1937.

In continuing gardenia canker studies initiated by R. P. White at this station, it has been observed that a large percentage of infection starts at the leaf joints on the base of the cuttings after they are set in the rooting medium. The practice among commercial growers is to remove the lower leaves with a sharp knife before embedding the cuttings. These freshly cut surfaces furnish an excellent point of entrance for the fungus and are important foci for later infection.

Twelve-week-old rooted cuttings of the variety *veitchii,* with stem cankers, are shown in figure 1. The plant on the left shows 3 well-developed cankers located at the points where leaves were removed. Typical pycnidia and spores of *Phomopsis gardeniae* were visible in these cankers. Pure cultures of the fungus were obtained from each of the 3 cankers. The plant on the right shows a more extensive canker that apparently also started at the point where a leaf was removed.

This observation is in accord with that made by several commercial growers who have been unsuccessful in reducing the incidence of disease, even though they avoided unnecessary wounding of the plants during handling and cultivation, and kept the insect population at a minimum. Work is in progress to develop a wound dressing for protecting cuttings whose lower leaves are removed prior to being placed in the propagating bench.—P. P. PIRONE, Department of Plant Pathology, New Jersey Agricultural Experiment Station.

Chlamydospore production on artificial media by Urocystis gladioli.[1]—On February 8 of this year, H. W. Rankin, extension pathologist at this station, received for disease diagnosis some gladiolus bulbs from a grower at Fairview, Pennsylvania. This material was turned over to the writer for microscopic examination. Associated with the lesions were 2 or 3 *Penicillium*-like organisms and a smut, tentatively identified as *Urocystis gladioli* (Requien) Smith, until inoculation studies could be made to establish its true identity. This is believed to be the first report of the gladiolus smut from America.

Attempts were made to trace the origin of the smut, but our findings to date give us only the information that the bulbs had been grown in the above locality for the past 7 years.

Germination of the spore balls was undertaken by streaking them on potato-dextrose-agar plates. At the end of 12 hours germination was observed. In all observed cases it was akin to that of *Urocystis cepulae* Frost. (Fig. 1.)

In an attempt to free the smut spores from the ever-present *Penicillium,* dilution plates were made and individual spore balls were cut out of the agar and transferred to slants of potato dextrose agar on March 19. When these tubes were examined on March 27, several of them showed a *Penicillium* contamination, but 6 of them were free of this contaminant. These 6 tubes were alike in that the fungus present produced a reddish-brown prostrate

[1] Contribution No. 116 of the Botany Department.

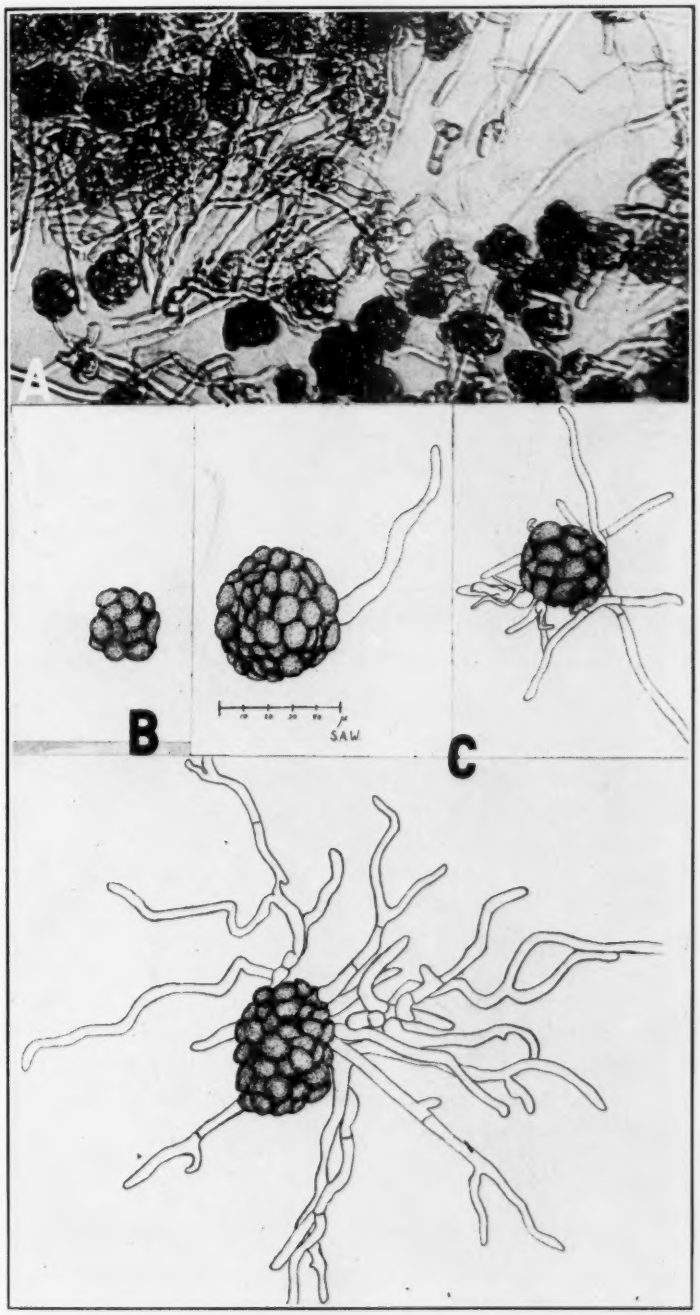

FIG. 1. A. Chlamydospores of *Urocystis gladioli* produced in a potato-dextrose-agar culture. B and C. Chlamydospores from a gladiolus corm. C shows stages in germination.

mycelium of uniform appearance. Mounts were made from these tube cultures and examined under the microscope. Chlamydospores of the *Urocystis gladioli* type were present in large numbers in each mount. Subcultures of these spores have been made. The spores from these cultures germinate in the same manner as do those from the corms and at this writing are growing nicely.

This phenomenon concurs with that observed by Kneip[2] for *Urocystis anemones* (Pers.) Winter. To the writer's knowledge these are the only 2 species of *Urocystis* with which it has been shown possible to grow the smut from spore to spore under saprophytic conditions.

Acknowledgments are due to Professor L. O. Overholts for the photographs and to my wife for the drawings.—C. C. WERNHAM, Department of Botany, The Pennsylvania State College.

[2] Kneip, H. *Urocystis anemones* (Pers.) Winter. Zeitschr. f. Botanik. 13: 289–311. 1921.

NOTICE TO MEMBERS OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

At the Indianapolis meeting, the Society instructed a committee to formulate a list of technical words for which uniform international usage is highly desirable and to draft definitions for them for consideration at the Richmond meeting. It is planned that those words and definitions surviving the Richmond discussion be proposed at Stockholm for plenary action. Lists of words preferably with definitions, are solicited from all members of the Society. Attention is drawn to Wilbrink's list, Amsterdam Congress v, 2, p. 204. See also Crane, E. J., Words and Sentences in Science and Industry. Science 86: Dec. 17, 1937. Your committee are content to deal with a relatively small number of words but will welcome from any source a "complete" system. They feel that words of Latin derivation should be given preference wherever possible and they particularly solicit definitions in several languages. Communications should be addressed to the chairman and to be most useful should reach him before Sept. 1, 1938.

JESSIE I. WOOD
NEIL STEVENS
DONALD REDDICK, *Chairman.*